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Helminthology and all branches of Parasitology*

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THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

THE SOCIETY meets once a month from October through May for the presentation and discussion of papers in any and all branches of parasitology or related sciences. All interested persons are invited to attend.

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Fifty Years—Fifty Volumes of the Proceedings of the Helminthological Society of Washington 1934–1983¹

The Society started the Proceedings of the Helminthological Society of Washington as its own Society-owned publication in 1934. The Society's earlier proceedings were published in *Science* (1911–1913) and the *Journal of Parasitology* (1914–1933). Now, after 50 years of this Society-owned Proceedings, a review of its evolution would appear to be of historical interest to the members and subscribers.

During the 50 years of publishing the Proceedings, it has had the developmental influence of only six successive Editors²:

Jesse R. Christie (1934–1947; 14 yr),
Edward G. Reinhard (1948–1951; 4 yr),
Gilbert F. Otto (1952–1965; 14 yr),
Francis G. Tromba (1966–1970; 5 yr),
Harley G. Sheffield (1971–1975; 5 yr),
A. James Haley (1976–1983; 8 yr).

These six are the men who shaped the Proceedings, all facing special problems organizationally, editorially, financially, and otherwise, yet who are the architects of what seems today to be a highly disciplined, respected, international scientific journal.

When free publication was available to members only, the Society gained many members from all parts of the United States and from all over the world. The result, in retrospect, seems to have been good for all concerned.

We shall look further into people who have handled the Proceedings, but circumstances seem to require that we preface these and other historical data with brief reference to the aforementioned precursors of the Proceedings of the Helminthological Society of Washington.

It is evident that the Society had already matured considerably when the Proceedings was inaugurated. Minutes of the first fifteen meetings had been published in eleven issues of *Science*. Minutes or notes on the 16th through the 156th meetings were published in 32 issues of the *Journal of Parasitology*, Volumes 1 through 20. These reports were true proceedings of the meetings, including reference to and gist of papers presented. They were prepared and submitted by the Secretary, for meetings 1 through 148 (1911–1932). These were essentially informal notes, unreviewed and unedited. They were digests of what was said or, more accurately, what the secretary thought the various speakers had said. It is worthy of note that the succession of secretaries included Maurice C. Hall, Jesse R. Christie, and Willard H. Wright—a very creditable trio. The only change in 1933 (meetings 149–156) was the formal listing of speakers and titles with formal abstracts. These abstracts were notes usually, but not always, prepared by the

¹ Published with the support of the Brayton H. Ransom Memorial Trust Fund.

² The Editor-Elect (1984–1988) is J. Ralph Lichtenfels.



Christie (1889-1978), first Editor (1934-1947).
Photo by George F. Weber.



Reinhard (1899-1958), second Editor (1948-1951).

speaker at the request of the secretary (L. A. Spindler). Thus these continued to be true, and perhaps more authentic, records of the proceedings of the meetings prior to the publication of the Society-owned Proceedings. Both for the record and for the immediate use of the interested reader, references to the published record of these earlier meetings are provided (Table 1). This table was prepared some years ago by Miss Edna M. Buhner, who served diligently as the Society's Secretary-Treasurer for 38 busy years (1934-1971) (i.e., beginning the year that the Proceedings was born). This informative tabulation was distributed to a few early members of the Society, but not heretofore published.

Meanwhile, the ownership of the Journal of Parasitology passed from the venerable Henry Baldwin Ward to the American Society of Parasitologists and the editorship from Ward to William Walter Cort, an exceptionally respected active member of the Helminthological Society of Washington, the American Society of Parasitologists, and other scientific societies. Details of considerable interest and pertinency are given in the Special Fiftieth Anniversary Number of the Proceedings of the Society (Historical Resume 27:242-260, 1960). Both Ward and Cort complained about the HelmSoc proceedings as submitted on grounds of content (e.g., articles on "nemas," obituary information, cost of printing, lack of editorial review, and other matters). In a nutshell, both Ward and Cort, with great pride and dedication, acted predictably as conscientious editors. Ward was adamant and apparently ready to give up the Journal of Parasitology rather than compromise. Cort apparently felt that the Journal would be a good official organ for the American Society of Parasitologists, which was by then well established, and that the HelmSoc proceedings, because of their variety, could be an asset to



Third to seventh Editors, left to right (with terms of offices): Otto (1952-1965), Tromba (1966-1970), Sheffield (1971-1975), Haley (1976-1983), Editor-Elect Lichtenfels (1984-1988). Photo courtesy Ronald Fayer, USDA.

the Journal and would be welcomed if dressed up a bit. He also stated during debate regarding the publication of its own Proceedings by HelmSoc that the latter might do well or better to consider paying costs of publishing in the Journal of Parasitology.

Nevertheless and despite difficult times—there had yet been no significant recovery from the “Great Depression” of 1929—the more mature minds of HelmSoc (Cort, Hall, Schwartz, Cram et al.) did not prevail, and strong protagonists for independent publication (Dikmans, Chitwood et al.) won the day! Regardless of whether this was good or bad then, at 24 years of age the Society, HelmSoc, launched its own journal, the Proceedings. Now, matured at 50 years, the Proceedings has grown into an internationally recognized journal of parasitology.

Number One of Volume One was issued in April 1934. It was composed of 34 papers on 24 pages. Among the authors were familiar names such as Fallis, Hall, Mueller, Wright, Steiner, Price, and McIntosh. The minutes noted the deaths of two distinguished Honorary Members: Constantin Janicki of the University of Warsaw and Friedrich Fülleborn of the Institute für Schiffs und Tropenkrankheiten. In contrast, issue Number One of Volume 50 (1983) contains 18 papers and 4 research notes, with some miscellanea, on 185 pages. Short research notes have been succeeded by larger primary papers. Specifics are not significant, particularly without detailed analysis, but, over all, a very significant change has taken place in relation to the proceedings of the Society’s meetings. The published notes of the Society were, in fact, a record of the meetings, while the official journal—the Proceedings—beginning in 1934, has not been in any sense a record of proceedings of the Society. There seems to have been a rather sudden evolution of the journal content notwithstanding the persistent, probably subconscious, repetition of the

Table 1. Meetings of the Helminthological Society of Washington, 1911-1933.

Meeting	Place of publication	Vol.	No.	Pages	Publ. date
1, 2	Science N.S.	33	840	197-198	1911
3	Science N.S.	33	848	510-512	1911
4	Science N.S.	33	850	590-592	1911
5, 6	Science N.S.	33	860	974-976	1911
7-9	Science N.S.	35	901	553-556	1912
10	Science N.S.	35	903	635-636	1912
11	Science N.S.	35	906	756	1912
12	Science N.S.	37	941	78	1913
13	Science N.S.	37	944	197-198	1913
14	Science N.S.	37	952	498-499	1913
15	Science N.S.	37	954	577-578	1913
16-20	J. Parasitol.	1	1	52-53	1914
21	J. Parasitol.	1	2	106	1914
22-24	J. Parasitol.	1	3	154-155	1915
25, 26	J. Parasitol.	1	4	200-203	1915
27	J. Parasitol.	2	2	93-95	1915
28, 29	J. Parasitol.	2	4	195-200	1916
30-38	J. Parasitol.	6	4	198-201	1920
39-44	J. Parasitol.	7	2	95-102	1920
45-50	J. Parasitol.	7	4	186-201	1921
51-53	J. Parasitol.	8	2	92-96	1921
54-61	J. Parasitol.	9	1	35-49	1922
62-65	J. Parasitol.	9	4	236-246	1923
66-70	J. Parasitol.	10	4	208-217	1924
71-76	J. Parasitol.	11	2	91-122	1924
77-82	J. Parasitol.	11	4	217-230	1925
83-89	J. Parasitol.	12	2	105-115	1925
90-92	J. Parasitol.	12	3	177-181	1926
93-96	J. Parasitol.	13	1	83-90	1926
97-103	J. Parasitol.	14	1	54-72	1927
104-107	J. Parasitol.	14	3	197-203	1928
108-110	J. Parasitol.	15	1	67-73	1928
111-115	J. Parasitol.	15	3	217-224	1929
116-120	J. Parasitol.	15	4	281-293	1929
121-126	J. Parasitol.	16	3	158-169	1930
127-130	J. Parasitol.	17	1	52-58	1930
131-134	J. Parasitol.	17	4	230-236	1931
135-139	J. Parasitol.	18	1	44-56	1931
140-143	J. Parasitol.	18	4	303-311	1932
144-148	J. Parasitol.	19	1	83-94	1932
149-151	J. Parasitol.	19	3	242-256	1933
152-154	J. Parasitol.	20	1	62-79	1933
155, 156	J. Parasitol.	20	2	97-114	1933

Published through the courtesy of Miss Edna M. Buhrer.

policy (on the inside of the front cover) that the Proceedings were intended as a record of papers given at the meetings. The phrase "*a medium for the publication of the notes and papers presented at the Society's meetings*" persisted for over 30 years in the standing announcement on the inside of the front cover. It was not deleted until 1965 (Volume 32), when shifting the identity of the Editorial Com-

mittee from the front cover to the inside of the front cover necessitated some condensation.

The records in the Proceedings of the Society's scientific program are confined to the Recording Secretary's minutes on two to four pages, usually in the July (No. 2) issue. These include a list of speakers and titles without any further reference to the subject matter. The few papers that may have been later published in the Proceedings are without any cross reference to the presentation at a meeting. Awards and other nontechnical activities of the Society will usually be found on a few pages in either of the two issues each year (each volume). Exception to this was the Special Anniversary Number (No. 3) issued in December (27:209–288, 1960). This contained the full program of the special meeting (Saturday, October 9, 1960) celebrating the fiftieth anniversary of the Society. It included the unabridged text of the papers presented by the invited speakers, and a comprehensive decade-by-decade history of the Society.

Two other special issues were also published. Neither of these had any relationship to the Society's programs. In both cases the total costs of publication were borne by the two agencies involved. Both issues were mailed routinely to all members and nonmember subscribers.

The first of these, Basic Research in Malaria, was included with Volume 39 (1972) as an unnumbered special issue in November, containing xiii + 582 pages. It was the official report of an international panel workshop at Walter Reed Army Medical Center, July 7–9, 1972. Although both the Editor (Elvio H. Sadun) and the Assistant Editor (Arthur P. Moon) were members of HelmSoc, most of the 75 panelists (speakers and authors) were not.

The other unnumbered issue was with Volume 42 (1975) in December, and consisted of 92 pages including 207 illustrations. It was a monograph by one of our members (J. Ralph Lichtenfels, our Editor-Elect) on Helminths of Domestic Equids.

With the above exceptions, most, if not all, of the papers had one or more members as author or authors. The policy of charging nonmembers the full cost of publication is associated with the increase in nonresident members, including foreign members. Membership in the Society from the beginning has been international, but foreign members have increased with the publication of the Proceedings. Many of the authors do not routinely attend the meetings and some have never attended any of the meetings.

It would be remiss to neglect the technical aspects of the Proceedings and its production. From an almost personal production by the first Editor, it has progressed to production by the most modern techniques. This has not been a sudden change, but a gradual improvement. The Society has been fortunate in its relationship with Allen Press for nearly two decades. As improved supplies and equipment became available, they were promptly utilized. The Proceedings has been shifted from one column to two columns and back to one column as newly developed techniques offered improvements with these successive formats. We are particularly pleased with the current easily readable text and detailed accuracy of the illustrations.

The source of funds to publish the Proceedings over the years has been a function of both the Society's reserves and the immediate national and international economy. Initially authors paid the cost of publication. Fortunately the costs were

Table 2. Proceedings of the Helminthological Society of Washington. Two numbers (January and July) per volume.*

Volume	Year	Pages	Volume	Year	Pages
1	1934	67	26	1959	168
2	5	113	27	1960	208 + 80*
3	6	87	28	1	224
4	7	84	29	2	224
5	8	76	30	3	304
6	9	106	31	4	304
7	1940	104	32	5	260
8	1	60	33	6	220
9	2	88	34	7	271
10	3	95	35	8	280
11	4	74	36	9	297
12	5	72	37	1970	256
13	6	59	38	1	278
14	7	89	39	2	283 + 595*
15	8	82	40	3	308
16	9	118	41	4	268
17	1950	134	42	5	193 + 92*
18	1	142	43	6	244
19	2	128	44	7	248
20	3	132	45	8	284
21	4	126	46	9	301
22	5	135	47	1980	287
23	6	172	48	1	269
24	7	152	49	2	340
25	8	146	50	3	

* Exceptions: Three issues: 1960, the Society's Fiftieth Anniversary issue (December, Vol. 27, No. 3, 80 pp.); 1972, a special unnumbered issue on Basic Malaria (November, Vol. 39, xiii + 582 pp.); 1975, a special unnumbered issue on Helminths of Domestic Equids (December, Vol. 42, 92 pp.). See page 191 for details.

limited, because the first Editor personally performed much of the labor. By the second volume, authors' charges were eliminated (for members). Eventually (Vol. 17, 1950), Society dues were reduced from \$5.00 to \$4.00; nonmember subscriptions remained at \$1.75 domestic and \$2.00 foreign. However, in 1955 (Vol. 22) diminishing Society reserves caused the solicitation of contributions from member-authors whose papers "*are inordinately long or have excessive tabulations or illustrations.*" Ten years later (1965, Vol. 32) the dues were raised to \$6.00 and the nonmember subscriptions were also increased. Despite these and subsequent increases in both membership dues and subscriptions, increasing costs have necessitated mandatory page charges. Currently, member-authors are charged for half the cost of printing, with no free pages.

The Brayton H. Ransom Memorial Trust Fund has made annual contributions, albeit nominal, to the Proceedings since the fund was established. During the 1980's it has also provided funds to cover all or part of the page charges for some authors.

At this point we should consider in more detail the editorial personnel, i.e., the people identified with the Proceedings. It has not become the highly respected international journal in parasitology, including the growth from 67 pages to over 300 pages per volume (Table 2), without the dedicated efforts of many scientists.

They have served the Society and the scientific world without any compensation beyond the satisfaction of doing the job well. Table 3 supplements and updates the lists of Editors and Editorial Committees previously published (24:157, 1957; 27:259, 1960). The names and years of service illustrate the plethora of high-caliber manpower available and used. The 72 scientists listed have contributed over 400 man-years of service. The individual periods of service ranged from 1 to 46 years, with an overall average of more than 6 years. If the time spent averaged no more than 5 days per year, their investment at \$100/day would amount to \$200,000, i.e., over \$4,000 per volume (i.e., year). Nor does this complete the picture. Many specialists who are not acknowledged in these published lists remain anonymous, unsung "heroes."

In general the Editors of the Proceedings have been persons of sound and broad biological backgrounds, well established and respected in their particular disciplines, and especially admired by their peers for personal traits and achievements, including their interest in and understanding of people. Each appears to have been motivated by ideals of service and a keen concept of fairness and impartiality. None has been extremist or controversial. The first two (Christie and Reinhard) were not even "orthodox" parasitologists, if one is permitted to use that expression (i.e., they did not study parasites of man and animals). One was primarily concerned with free-living nemas and nemic parasitism of plants. The other was concerned with parasitic crustacea and the phenomenon of parasitic castration. Both were leaders in their fields. Later Editors, also leaders of unquestioned stature, were more orthodox parasitologists. Each of them also contributed uniquely to the development of the Proceedings.

The first Editor was Jesse R. Christie (1934–1947); the other members of the Editorial Committee were Otto, Price, Ewing, and Spindler, the latter *ex officio* as Secretary of the Society. This Committee remained intact with two variances throughout Christie's 14 years of conservative, superlative editorship. The variances were (1) the succession of Society Secretaries after Spindler, mandated at that time by the Bylaws, and (2) the appointment of Willard H. Wright to succeed Henry E. Ewing in 1947. This was the only appointment made by the first Editor after the original Committee was established, and it came about because of the failing health of Ewing (18:141–142, 1951). It may be computed that 15 committeemen including the Editor gave a total of 70 years, or an average of 4.7 years of service each, during Christie's 14 years as Editor.

Christie was selected as the first Editor with some acclaim. Although no reasons were articulated, so far as known, he was eminently qualified. He was well liked, highly respected, and a mature, recognized scientist (46:158–159, 1979; J. Parasitol. 64:1038, 1978; Nematol. Newsl. 24, June 1978). He represented the plant nematode area, a circumstance that seemed to signal an end to the questions that arose about publication of "nema" papers in the Journal of Parasitology. He took no part in any of the controversies preceding establishment of the Proceedings. Among other attributes, he was a hobbyist in printing and publishing. There was some conviction, among members at that time, that he may have set type and even had a hand in the actual printing of the first number or two.

His Editorial Committee was representative and noncontroversial. Altogether, it appears, in retrospect, that the Society initiated its Proceedings in troublesome, Depression times, yet got off to a good stabilizing start.

Our second Editor, Edward G. Reinhard (Vols. 15–18, 1948–1951), was also unquestionably remote from the earlier editorial divergencies. Furthermore, his selection as Editor reemphasized that the Society is not wedded to the parasitic helminths, despite its name. His interests had been variously termed as ecology, animal ecology, plankton, limnology, and entomology. He listed his interest more inclusively as invertebrate zoology. Neither helminths nor parasitology had been depicted as one of his interests. Nevertheless, even before launching on his doctorate program, he published his findings on the parasitic wasp, *Nysson hoplisi-vora*. Later he became an authority on the biology of rhizocephalan crustacea, genera *Sacculina* and *Peltogaster*, which are parasites of higher crustaceans (i.e., decapod crabs). This included reports on parasitic castration, a hyperparasitic amoeba in rhizocephalans, and contributions on nematodes, trematodes, and acanthocephala (25:73–77, 143, 1958).

What Reinhard brought to the Society and its Proceedings, as Editor, was a broad background in biology and an innate dedication to accuracy, the attributes of a self-effacing, dedicated scientist and teacher. He instituted peer review of *all* manuscripts. In that sense the Proceedings joined the group of recognized scientific journals. He instituted two other changes that upgraded both the Proceedings and the Society. He added distinct covers on the former and published the membership of the latter. Although he relinquished the editorship on completion of Volume 18 (1951), Reinhard continued to serve effectively on the Editorial Committee through Volume 25, until his death in 1958.

Third editor³. When the “independent” Proceedings came to Volume 19 (1952), the time apparently was appropriate and safe to select an Editor whose capacities and scientific stature were never in question, despite being pretty much of a “regular” parasitologist and, of course, one who could get the job done effectively, with fairness to all. Indeed, the man selected was a part of it all from the beginning, historically knew it all, had seen it all, knew everybody, and everybody knew him. In passing, it should perhaps be emphasized that traits of a kind needed for Editorship were also fortunately found in all other Editors of the Proceedings; these included not only traits already mentioned, but also those such as breadth of background and experience, scientific acumen, concepts of fair and effective administration, and interest in people. The man selected in 1952 was the redoubtable, inimitable Gilbert Fred Otto.

Otto expanded and annualized indexing, improved format, and affixed on the cover the year of expiration of terms of members of the Editorial Committee, but generally followed the constitutionally prescribed five-man Editorial Committee. He also moved the posting (Vol. 32, 1965) of Editorial Committee members to the inside of the front cover in order to start the table of contents on the front cover. In Volume 32 (1965), he also shifted from a single column on a page to take advantage of the resulting savings available with the two-column format. He was also the first to increase the size of the Editorial Committee, from 5 to 6, although temporarily. In fact, the personnel of the Committee remained the same until Clark P. Read was named of necessity to succeed Reinhard in 1958 (Vol.

³ The account of the third Editor (G.F.O.) was written exclusively by the first author (A.O.F.) of this report, i.e., without editing by the second author. — The Editor.

25). Indeed, only two (Olivier and Taylor) of the original "Otto Committee" had not served on the Editorial Committee earlier. Later (1962), A. James Haley replaced Read. Although still later (1963) Allen McIntosh replaced Olivier and Christie (1964) replaced Taylor, these individuals were not new to the Editorial Committee. As of the end of Otto's 14 years of outstanding Editorship, involving 71 editorial man-years, as it were, only 5 "new" people became involved on the Editorial Committee (Read, Olivier, Taylor, Haley, and Weinstein, the last yet to be mentioned); thus 10 members served an average of 7.1 man-years each.

By all standards, Otto had been wholly conservative. Only in his last year as Editor (1965) did he vary from the fixed and established standard procedure. That year he made a combination of moves (probably in violation of the Bylaws) that, in retrospect, seemed to have opened Pandora's Box! He named Haley to Assistant Editorship, replaced Haley on the Editorial Committee with P. P. Weinstein, and by these moves put six members on the Editorial Committee. Predictably, no one challenged these moves, although the next Editor (Tromba) dropped the office of Assistant Editor but eventually increased the membership of the Editorial Committee to 15. In general, as previously mentioned, editors were given a free hand and the Bylaws, although enormously useful, even indispensably so, were rarely considered as instruments designed to thwart either obvious progress or the clear will of the majority.

Later Editors exploited these precedents, not without reason and loyalty to purposes to be served in behalf of the Society, but with rather free, if unexpressed, approval of their actions.

One particularly interesting story pertains to these developments. It concerns the appointment of A. James Haley to the Editorial Committee (1962) and to Assistant Editorship (1965) and it concerns later developments, not to mention the fact of Otto's unique service to the Editorial Committee from Volumes 1 through 50, or the fact that Haley was destined to become Editor in 1976, being currently the sixth and last Editor of the first half-century of the Proceedings. It is likely of some significance that Haley had been a student of Otto's at Johns Hopkins University and that Otto, while Editor, had regarded Haley as a good prospect for future Editorship. To make a long story short, the Helminthological Society on the occasion of its Seventieth Anniversary awarded a unique special service citation to Otto. The presentation was made by none other than the Editor (Haley) to the only one who had "continuously served" from the beginning on the Editorial Committee. No one demurred. No one could, but the fact is that Otto was and is the only individual who has served continuously from the beginning on the Editorial Committee, *except* for the first 4 years of, yes, Haley's Editorship! In eight years (1976–1983) Haley has appointed some 25 new members to the Editorial Committee; some 27 were dropped when their terms expired. Among the latter was Otto; he was, as mentioned, reappointed in 1981! His reappointment was recommended by Editor Haley during presentation of the special service award. Otto's service during the period when he was not technically a member of the Committee was probably more significant than at any time before or after; he clearly served during that term as advisor and back-up editor, as well as in the usual editorial review and service capacities. It seems fair to say that most members of HelmSoc regarded Otto as a kind of Life or Honorary Member of most everything. That Otto was unwittingly not reappointed to the Editorial Committee in 1976 is now quite clear; it is even more clear that there is no basis

in anyone's mind to question the accuracy of Otto's "continuous service" to or on the Committee!

The fourth editor, Francis G. (Frank) Tromba (Vols. 33–37, 1966–1970) is also an "orthodox" parasitologist. He was, until his recent retirement, a career staff member of the internationally recognized prestigious unit in the United States Department of Agriculture, the Beltsville Parasitology Laboratory (earlier the Zoological Division of the U.S. Bureau of Animal Industry and currently the Animal Parasitology Institute). Tromba was the first Editor from this laboratory in which the primary stimulus developed for the Society's own Proceedings. There is no evidence that the future of the papers submitted for publication were or are appreciably affected by the Editors' research interests or institutional affiliations. Nevertheless, like his immediate predecessor, Tromba's primary interest was in experimental investigation of parasites of man and/or animals. The disciplinary affinities of the papers have changed. They continue to represent the broad and varied interests of the membership. The decline in taxonomic papers reflects a continuing diversion of papers on "nemas" to the newer *Journal of Nematology*. These and related papers have been more than replaced by reports of experimental studies.

Tromba added the preceding Editor and Assistant Editor to the previous Editorial Committee, bringing it to six in addition to the Editor. In his third year, he enlarged the committee to nine and the next year to 14 to better provide for peer review of the widely diverse disciplines covered by the papers submitted for possible publication. While maintaining the high standards of the published papers, the Proceedings increased in size to an average of 260 pages per volume in his five-year term, with a maximum of 297 pages in Volume 36 (1969).

The total man-years of effort was 54 for his five-year term, an average of 11 per year. Shifting the appellation Editorial Committee to Editorial Board in Volume 36 (1969) was the Editor's (Tromba's) acknowledgment of the increasing importance to the Proceedings of these dedicated scientists.

The fifth Editor, Harley G. Sheffield (Vols. 37–42, 1971–1975) came from another institution and represented still another discipline in parasitology. His international recognition centered on the biology of medically important protozoa at the Institute of Allergy and Infectious Diseases of NIH and earlier in a pharmaceutical company (Parke-Davis). He continued the 14-person Editorial Board. In his five-year term, 21 scientists served for a total of 75 man-years, including the Editor (and the additional Assistant Editor for three years), an average of 3.6 man-years. During his term it became necessary to assess member-authors \$10 a page, starting with Volume 40 (1973). Nevertheless, the five volumes continued at the previous level, averaging 266 pages; the largest (308 pages) was that year. The trend from taxonomy to the biology of parasites continued. Of 143 papers published during his five-year term, only 24 (17%) were descriptions of new species. The 50% shift in editorial personnel, 21 scientists serving in five years on the 14-member board, again illustrates the wide diversification of the papers. The continuing high caliber of the peer reviews contributed to the continuing excellence of the Proceedings, which he maintained. To help maintain these standards he appointed an Assistant Editor, Guillermo Pacheco, for three years (Vols. 39–41, 1972–1974).

He also introduced unnumbered special issues that added significantly to the value and prestige of the Proceedings. The first (Vol. 39, November 1972, xiii +

582 pp.) was the official report of a panel on Basic Research in Malaria. The second (Vol. 42, December, 92 pp.) was a monograph on the Helminths of Domestic Equids (see p. 191 for details).

The last of six Editors during the first half-century, A. James Haley, has probably handled more manuscripts and a greater diversity of subject matter than any of his predecessors. He has done it with efficiency, aplomb, and rigid adherence to high standards. He streamlined the Editorial Committee without delay, setting up a committee of 15 members, in addition to the Editor, each for three-year terms of office and all terms staggered in such manner that terms of five members (one-third) expired each year. There are apparently no stipulations against reappointment, nor any specific encouragement for it. This is in accord with the policy of HelmSoc that members of the Editorial Committee serve at the will of the Editor. Haley has been conservative; he seems to have followed his organizational plan and has fired no one, but has reappointed few. He has exercised considerable latitude in the 35 necessary replacements of members whose three-year terms expired over an eight-year period. There were 25 new appointees to the Editorial Committee. Ten (28%) enjoyed reappointment; three out of four were replaced by new people when their terms expired. This circumstance can best be ascribed to the burgeoning technology and scientific knowledge. The Editor had to find new reviewing expertise and was doing only what he found was necessary.

He took advantage of further technical improvements to return to the single-column format in Volume 46 (1979), again with financial savings.

The second half-century of the Proceedings (Vol. 51, 1984) starts with the seventh editor, J. Ralph Lichtenfels. His appointment brings the management of this journal back to the Animal Parasitology Institute (USDA), back to what many of us consider the primary domicile of HelmSoc, certainly whence came the impetus that resulted in the Proceedings. Lichtenfels, like the first Editor (Christie), is an acknowledged taxonomist, although his taxonomic interests are different. There is little if any indication in the first 50 volumes that scientific expertise of the Editor has materially influenced the content of the Proceedings. Taxonomists are, at least, punctilious in their insistence on accuracy. This is an important attribute for an Editor and augurs well for the continued high standards set by his predecessors. The disciplinary inclusions can be expected to follow the evolutionary changes in the research and the resulting expertise of the authors.

In substance, this is the saga of the Proceedings of the Helminthological Society of Washington. It contains much that is undocumented, also some that is naught but reminiscence—the way things seem to have been based on recollections of individuals whose memories on this fiftieth year of publication may be faulty, even misleading. In general, however, there has been verification, supplemented by reference to supplementary reading on conjectural and personal interpretations.

If our world situation permits, our Proceedings can be said to face a growing, demanding, successful future.

AUREL O. FOSTER
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Zoology Department
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College Park, Maryland 20742

In Memoriam

Leo Albert Jachowski, Jr. August 17, 1918–June 8, 1983

Dr. Jachowski, Comdr. U.S.N. Ret., a native of the Baltimore–Washington area, was an internationally recognized parasitologist. His education after high school was at Benjamin Franklin University, University of Maryland, University of Michigan (B.S. 1941, M.S. 1942), and The Johns Hopkins University (Sc.D. 1953). While a Ph.D. candidate at the University of Michigan, in 1943 he joined the Navy as an Ensign. He retired from the Navy in 1964, with the rank of Commander. He then joined the faculty of the University of Maryland as a full-time Professor of Zoology, from which he retired in 1980.

Jachowski's major research interest was the biology and control of arthropod-borne disease, but also included schistosomiasis. Among other things, he contributed to our knowledge of immunity to, and immunodiagnosis, chemotherapy, and control of, malaria, filariasis, and schistosomiasis. His field studies in Alaska, Guatemala, and Samoa included the epidemiology, chemotherapy, and control of filariasis (elephantiasis); insect repellents; and epidemiology of schistosomiasis. One of his outstanding contributions was the demonstration that aperiodic *Wuchereria bancrofti* in the South Pacific was transmitted primarily, if not exclusively, during daylight in the "bush" in contrast to the nocturnal, domestic (house) transmission of the periodic strain of this parasite elsewhere in the tropics.

His teaching experience included the University of Michigan, School of Hygiene and Public Health of The Johns Hopkins University, and the Medical School of the University of Puerto Rico in addition to 16 years in the Zoology Department of the University of Maryland. At Maryland he taught an upper division course in parasitology and various related graduate courses. Thirteen students received the M.S. degree and 11 received the Ph.D. under his tutelage. In addition, he served on numerous Department and University Committees, was Director of Research (1966–1967), Director of Graduate Studies (1968–1971), Assistant Department Head (1965–1967, 1970–1971), and Acting Department Head (1968–1970).

Jachowski was a member of the American Heartworm Society, American Microscopical Society, American Mosquito Control Association, American Society of Parasitologists, American Society of Tropical Medicine and Hygiene, International Society of Filariasis, Royal Society of Tropical Medicine and Hygiene, and the Washington Association of Tropical Medicine, in addition to the Helminthological Society of Washington. He joined HelmSoc in 1950 and was elected Life Member in 1981. He served the Society on many active committees and as Recording Secretary (1960), Vice President (1964), and President (1965). He received the Society's Anniversary Award in 1976.

Among other awards and military decorations he received the Bailey K. Ashford Award from the American Society of Tropical Medicine and Hygiene in 1952 and the Isaac Gonzales-Martinez Award from the Puerto Rican Bilharzia Committee in 1959.

Leo Jachowski is survived by his wife, Virginia, 4011 Prospect St., Kensington, Maryland; two sons, Richard L., of Fairfax, Virginia, and Peter R., of Cody, Wyoming; two daughters, Julia Devine, of Kennedyville, Maryland, and Jane Wallace, of Mt. Vision, New York; his mother, Elsie S., of Washington, D.C.; a brother, Robert A., of Rockville, Maryland; a sister, Frances Van Winkle, of Knoxville, Tennessee; and two grandsons.

See also Trop. Med. Hyg. News, Vol. 32, No. 4, August 1983.

GILBERT F. OTTO

The Brayton H. Ransom Memorial Trust Fund

A. MORGAN GOLDEN

Nematology Laboratory, Plant Protection Institute,
BARC—West, Beltsville, Maryland 20705

This Trust Fund was established in 1936 to honor and perpetuate the memory of an outstanding U.S. scientist whose career was suddenly ended by his early death in 1925. (See obituaries especially in *Journal of the Washington Academy of Sciences* 15(8), Nov. 4, 1925, and in *Science* LXII(1606):319–320, Oct. 9, 1925.) The present Board of Trustees feels it appropriate and timely to bring to the attention of our scientific community some details and information about this Fund, beginning with republication of the legal document establishing it, published in the *Proceedings of the Helminthological Society of Washington* 3:84–87, 1936.

Whereas Dr. Brayton H. Ransom has rendered valuable services to humanity by his contributions to the study and control of parasites; and

Whereas the Helminthological Society of Washington deemed it fitting that the memory of Dr. Ransom should be perpetuated, and in furtherance of this purpose appointed a committee composed of Ch. Wardell Stiles, Maurice C. Hall, Eloise B. Cram and W. W. Cort to solicit funds to be used to establish a suitable memorial; and

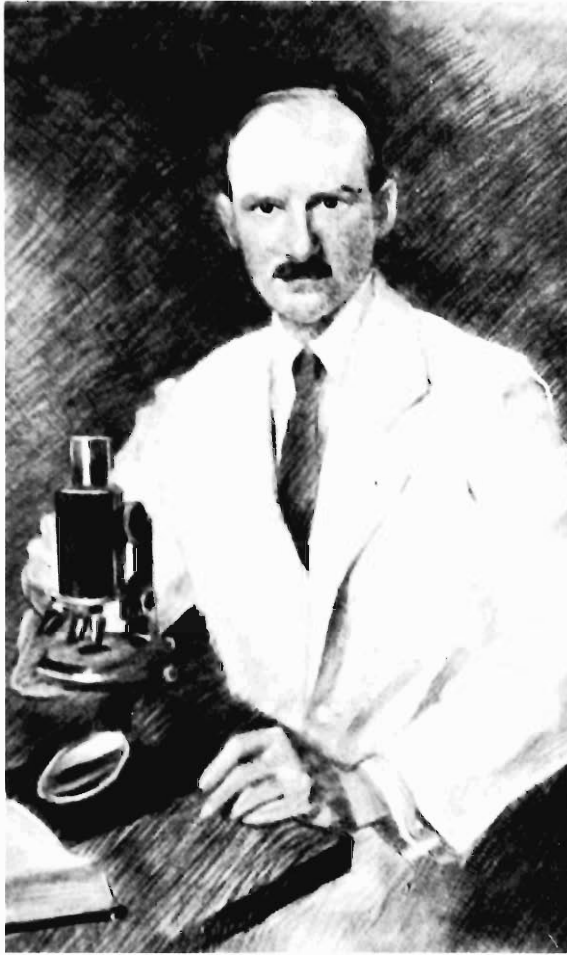
Whereas friends and colleagues of Dr. Ransom, residing in all parts of the world, have contributed as original donors a total sum of \$1,020.93 with the understanding that the contributions should be used to create a memorial in honor of Dr. Ransom, said original donors being as noted on the attached list.

And whereas, at the time of soliciting funds no definite form of memorial had been decided upon, but suggestions were solicited from the original donors and contributions were received with the understanding that the nature of the memorial to be established would be dependent on the total sum collected, keeping in mind the wishes of a majority of the donors; and whereas there has been no substantial agreement as to the form that this memorial in honor of Dr. Ransom should take except that it should be of a kind to encourage the study and advance of the Science of Parasitology and related sciences;

NOW, THEREFORE, in order to establish such a memorial, the present committee in charge of said funds nominated in October, 1935, consisting of G. Steiner, chairman, Paul Bartsch, Eloise B. Cram, G. Dikmans and G. F. Otto, as members, joined by M. C. Hall, C. W. Stiles and W. W. Cort, the surviving members of the original committee with the exception of Eloise B. Cram who is on the present committee, at the direction of the Helminthological Society, hereby gives, transfers, assigns and sets over, and delivers to Eloise B. Cram, G. Dikmans, G. F. Otto, E. W. Price and G. Steiner, TRUSTEES, named by the Helminthological Society to act as such, the sum of one thousand two hundred ninety-nine dollars and 91 cents (\$1,299.91), (contributions paid \$1,020.93, interest accrued \$290.73, expenses to date \$11.75), representing all the funds collected plus interest thereon up to the present date, minus expenses to date, IN TRUST, HOWEVER, for the purposes and uses described below; and the above named Trustees in consideration of the foregoing, hereby accept these funds subject to these said trusts and uses, and agree to carry out the terms of this Trust.

1. This trust fund is to be known as the BRAYTON H. RANSOM MEMORIAL TRUST FUND.
2. There shall be five Trustees, but any three of the Trustees agreeing on any subject matter *shall* have power and authority to transact any Trust business at any time and their action shall be binding on the Trust Fund and on all the Trustees. Trustees who are absent from a meeting shall be notified promptly of any action taken during their absence. In the event that some action by the Trustees is necessary and it is impossible to have a meeting, the Trustees may individually and separately and without consultation with other Trustees indicate their will in writing properly signed and witnessed and the action of three or more Trustees shall be binding on the Trust Fund and all Trustees.

3. Three or more of the Trustees shall have authority and power to draw checks and to indorse checks in the name of the Trust Fund, without including the names of the other Trustees, nor shall



Brayton H. Ransom (1879-1925).

it be necessary at any time for three or more of the Trustees to join the names of the other Trustees when executing any paper or instrument on behalf of the Trust Fund.

4. A majority of the Trustees shall name one of the Trustees to act as Secretary and Treasurer. The Trustee so designated shall keep a set of books showing investments, income, disbursements as well as all other information relating to the finances of the Trust. The secretary shall also keep a record of the meetings, the matters handled at such meetings and their disposition. He shall also record any action taken upon other affairs relating to the Trust Fund which may have been brought up and acted upon without having a formal meeting. These books and records shall be open to inspection by the other Trustees at any time during reasonable hours. In the event that the Trustees deem it more expedient to have separate individuals to act as Secretary and Treasurer, they may name one of their number to each post.

5. The Trustees, as soon as practical, shall take the funds herein transferred to them and invest the same in what appears to the Trustees to be a safe manner. No restriction is placed on the type of investment the Trustees may make, and they shall not be personally liable for mistakes of judgment. Any funds in their hands insufficient in quantity to be readily invested may be placed on deposit in a bank or other safe depository, in the name of this Trust, and shall be subject to withdrawal on the signature of three or more Trustees in the name of the Trust. If it is deemed expedient, a checking account may also be opened.

6. The Trustees of this fund shall use the income thereof (or so much of said income as they deem advisable) to encourage and promote the study of and advance of the Science of Parasitology and related sciences. But in NO case may they use the principal.

The principal of this Trust Fund shall be deemed to consist of: FIRST, the original contributions hereby transferred to the Trustees, but not the income such contributions have earned while in the hands of the committee, except such portion as may be specifically designated by the Trustees; SECOND, all subsequent contributions; THIRD, all past and future earnings of existing funds which shall have been set aside to be added to the principal by formal act of the Trustees.

The particular use to be made of the income shall be discretionary with the Trustees, as long as that use fulfills the general purpose of perpetuating the memory of Dr. Ransom and the advancement of the Science of Parasitology and related sciences. This use may take the form of presenting a prize or prizes at intervals, either in the form of money or a suitable medal for a notable contribution to this field of science, the publishing of worthy writings relative to this science, the subsidizing in part or in whole of a journal or other publication devoted in toto or in part to this field, the dissemination of knowledge, granting of scholarships or any other means which appear appropriate to the Trustees to accomplish the general and international purposes of this Trust. (The intent of the foregoing enumeration is to suggest possibilities and is not to be treated as a limitation.)

7. Because of the fact that the fund is small at present, it is suggested that not all the income be used for these purposes, but that a part of the income be kept and re-invested so that the principal may be increased, in order that the ultimate usefulness and importance of the fund may be increased. The Trustees are specifically authorized to accept further contributions.

8. The Trustees, upon creation of this Trust, shall notify all original donors of such action by publication in the Proceedings of the Helminthological Society of Washington, a copy of which is to be sent to all donors that can be reached.

9. The Trustees may meet whenever necessary but are required to meet at least once every year, and the use of the income for the past year shall then be discussed and determined and a report of their action published in the Proceedings of the Helminthological Society of Washington, or some other appropriate journal in the event the former ceases to be published. It is suggested that the Trustees set a fixed date for such annual meetings.

10. In the case of the death or resignation of a Trustee, a successor Trustee shall be elected by a majority of the remaining Trustees, it being intended that this body of Trustees shall be self-perpetuating.

11. The foregoing instructions are in the nature of instructions for the guidance of the Trustees and it is the intent of this instrument that in no case shall any party dealing in good faith with said Trustees in relation to the Trust Fund be obliged to see to the application of the funds or be obliged to see that the terms of this Trust are complied with or be obliged to inquire into the necessity or expediency of any act of said Trustees or be obliged to inquire into the terms of this trust agreement.

IN WITNESS WHEREOF WE HAVE HEREUNTO SET OUR HANDS AND SEALS AT WASHINGTON IN THE DISTRICT OF COLUMBIA, THIS 17TH DAY OF JUNE, 1936.

Witness the signature of W. W. Cort this 13th day of
June, 1936.

(Seal) Leonard O. Engle,
Notary Public.

District of Columbia,
June 17, 1936

Subscribed and sworn to before me this 17th day of
June, 1936, by all members of the previous and present
Committee and the Trustees of the Ransom Memorial
Trust Fund except W. W. Cort.

(Seal) W. E. Taylor,
Notary Public in and for
the District of Columbia.

Commission expires July 14, 1936.

COMMITTEE

Ch. Wardell Stiles
W. W. Cort
Maurice C. Hall
Eloise B. Cram
Paul Bartsch
G. Dikmans
G. F. Otto
G. Steiner

TRUSTEES

Eloise B. Cram
G. Dikmans
G. F. Otto
E. W. Price
G. Steiner

In the original version (Proc. Helminthol. Soc. Wash. 3:84–87, 1936), the above was followed by a list of 143 donors and pledges, containing the names of prominent workers worldwide.

* * * * *

The record of trusteeship over 47 years is a testimony of dedication and service of 13 Trustees who have served an average of 18 years. There have been four Chairmen (Steiner, Otto, Foster, and Golden) and five Secretary-Treasurers (Cram, Foster, Rozeboom, Golden, and Sheffield).

It is noteworthy that one individual has served continuously as a Trustee for 47 years (1936–1983), 17 of these as Chairman (or “President”). This individual also served (1935) on an antecedent committee of the Helminthological Society that was charged with formulating terms of a Trust and nominating an initial group of Trustees to be stewards of funds already collected (\$1299.91) for a memorial to Ransom (see Proc. 3(2), 1936, p. 84) and, of course, of all future funds added thereto, and the use thereof. That individual, not surprisingly, is none other than the indefatigable G. F. Otto. Dr. Otto did not have the good fortune to know Ransom personally; it may be recalled that Dr. Ransom died September 17, 1925, and that it was not until June 17, 1936, 11 years later, that the Society officially established the Trust (see Proc. 24(2), 1957, p. 145).

From time to time, the Trustees have reviewed potential uses of the modestly growing income from the Fund that might best perpetuate the memory of Dr. Ransom. It is probably as self-evident as it is a matter of record that the Trustees enjoy, at once, a great freedom of action in this regard and a heavy responsibility to do what seems best. With the very limited income to date, the Trustees appear, in retrospect, to have evolved a sound policy, namely a healthy blend of growth in principal and service to objectives that augurs a future that is best for both. Only now is the Fund in a position to permit the Trustees to entertain the best suggestion that a chance reader of this note may have. The Trustees’ judgment must be final, of course. Readers, and their friends, should know also that all donations to the Brayton H. Ransom Memorial Trust Fund are added to the principal of the Trust and are more than welcome.

Notwithstanding the aforementioned remarks, the Trustees have to date used the entrusted funds ingeniously, if not too creatively, but, we all hope, as Dr. Ransom would have wished. The first use of the interest from the Fund was, appropriately, token support of a struggling new journal, namely *The Proceedings of the Helminthological Society of Washington* (see Proc. 6(1), 1939, cover). This support has continued and has recently been increased somewhat, not only in support of the *Proceedings*, but also in support of publication of meritorious manuscripts by authors lacking institutional or other backing. The Trustees also established in 1960 (see Proc. 27(2), 1960, p. 208) an award of high honor, designated the Brayton H. Ransom Memorial Award, comprising a certificate but no monetary compensation. It has been awarded only once (see Proc. 27(3), 1960, p. 240), but may be awarded again. The Trust Fund also, on one occasion, loaned money to a needy parasitologist who temporarily signed over as collateral the cash value of a life insurance policy.

Although these comments are primarily historical, they nevertheless contribute information that may be of broader or special interest. The Brayton H. Ransom

Memorial Trust Fund is international, and its Trustees are receptive to serious suggestions about anything that it might do to better fulfill its objective and also contribute to peace and a better world.

* * * * *

In addition to the above note, and in commemoration of the fiftieth anniversary of The Proceedings of the Helminthological Society of Washington, it seems appropriate to bring to date for the record a further list of Officers and Trustees of the Brayton H. Ransom Memorial Trust Fund, continuing from those published heretofore in the Proceedings (24(2), 1957, p. 145; 27(3), 1960, p. 259) (Table 1). This article was prepared with the assistance of the present trustees, especially A. O. Foster.

Table 1. Officers and Trustees, Brayton H. Ransom Memorial Trust Fund, continuous from 1956 (see text).

1956–1967,	G. F. Otto, Chairman; A. O. Foster, Secretary-Treasurer; Edna M. Buhrer; K. C. Kates; Myrna F. Jones (Mrs. M. F. J. Robertson)
1967–1973,	G. F. Otto, Chairman; A. O. Foster, Secretary-Treasurer; Edna M. Buhrer; K. C. Kates; L. E. Rozeboom
1973–1974,	A. O. Foster, Chairman; L. E. Rozeboom, Secretary-Treasurer; G. F. Otto; Edna M. Buhrer; K. C. Kates
1974–1975,	A. O. Foster, Chairman; A. Morgan Golden, Secretary-Treasurer; G. F. Otto; Edna M. Buhrer; K. C. Kates; L. E. Rozeboom, Trustee Emeritus
1975–1979,	A. O. Foster, Chairman; A. Morgan Golden, Secretary-Treasurer; G. F. Otto; K. C. Kates; Harley G. Sheffield; L. E. Rozeboom, Trustee Emeritus; Edna M. Buhrer, Trustee Emeritus
1979–1982,	A. Morgan Golden, Chairman; Harley G. Sheffield, Secretary-Treasurer; G. F. Otto; A. O. Foster; K. C. Kates; L. E. Rozeboom, Trustee Emeritus; Edna M. Buhrer, Trustee Emeritus
1982–	A. Morgan Golden, Chairman; Harley G. Sheffield, Secretary-Treasurer; G. F. Otto; A. O. Foster; J. Ralph Lichtenfels; L. E. Rozeboom, Trustee Emeritus; Edna M. Buhrer, Trustee Emeritus

Report on the Brayton H. Ransom Memorial Trust Fund

Balance on hand, 1 January 1982	\$6,103.03
Receipts: Net interest received in 1982	784.70
	<u>\$6,887.73</u>
Disbursements: Grant to the Helminthological Society of Washington for 1982	50.00
Publication support	106.25
On hand, 31 December 1982	<u>\$6,731.48</u>

HARLEY G. SHEFFIELD
Secretary-Treasurer

Trustees of the Brayton H. Ransom Memorial Trust Fund

A. Morgan Golden, President	Aurel O. Foster
Harley G. Sheffield, Secretary-Treasurer	J. Ralph Lichtenfels
Edna M. Buhrer, Emeritus	Gilbert F. Otto
Lloyd E. Rozeboom, Emeritus	

Seasonal Dynamics of *Bothriocephalus acheilognathi* in Ambient and Thermally Altered Areas of a North Carolina Cooling Reservoir

WILLARD O. GRANATH, JR.¹ AND GERALD W. ESCH

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ABSTRACT: The seasonal dynamics of *Bothriocephalus acheilognathi* were studied in three arbitrarily established size classes of mosquitofish, *Gambusia affinis*, from thermally altered and ambient-temperature locations in predator-free areas of Belews Lake, a North Carolina cooling reservoir. At both stations, lowest prevalences and densities of the cestode were observed during summer months. Densities rose sharply in the fall and peaked by early winter, after which they began to decline. Recruitment of the parasite at each site was also seasonal, beginning in late spring and continuing into October of both years of the study. However, recruitment at the thermally altered site began about 2 wk sooner, lasted about 2 wk longer, and was interrupted for several weeks in late summer when water temperatures exceeded 35°C. Using a special sampling program, prevalence and density of *B. acheilognathi* were estimated for the overall mosquitofish population; estimated values closely approximated observed values. The seasonal changes in prevalence, density, and recruitment coincided with temperature changes. However, population dynamics were also apparently related to foraging strategies and prey availability, which varied seasonally and among size classes. Release from predation pressure allowed mosquitofish to expand their lateral spatial distribution. Mosquitofish exposed to copepods collected from limnetic areas of the reservoir became infected, suggesting that the spatial distribution of copepod intermediate hosts is not a factor in affecting the population biology of the cestode in Belews Lake.

Bothriocephalus acheilognathi Yamaguti, 1934 (= *B. gowkongensis* Yeh, 1955) was originally described from cyprinid fish in Japan. Dubinina (1982) has recently discussed the taxonomic position of *B. acheilognathi* and the closely related *B. opsalichthydis*. She is of the opinion that each is a distinct taxon; Dr. Hilda Ching (pers. comm. to Dr. Glenn L. Hoffman) expressed the opinion that both species may have been introduced into Europe and the U.S.; most recently *B. acheilognathi* was reported in Great Britain (Andrews et al., 1981). *Bothriocephalus acheilognathi* was introduced into the United States in grass carp in the early 1970's and since has become well established in the mid-south and southeastern United States. The spread of the parasite has been facilitated through its association with bait fish and aquaculture practices (Hoffman, 1980). During a routine survey of fish in Belews Lake, a large cooling reservoir located in the northern Piedmont area of North Carolina, *B. acheilognathi* was found in mosquitofish, *Gambusia affinis*.

The life cycle is typical of most bothriocephalids. Operculate eggs are shed into water via feces and, after an appropriate period of development, a motile coracidium emerges. Coracidia are consumed by and develop to a proceroid stage in several species of cyclopoid copepods, which, in Belews Lake, include *Cyclops bicuspidatus thomasi*, *Mesocyclops edax*, and *Tropocyclops prasinus*. The infected copepod is consumed by an appropriate piscine definitive host, where maturation to the adult stage occurs. According to Chubb (1981), *B. acheilognathi* has an

¹ Present address: Department of Zoology, 730 Van Vleet Oval, University of Oklahoma, Norman, Oklahoma 73069.

unusually large range of definitive hosts, having been reported from more than 40 species.

An understanding of the population dynamics of many host-parasite systems is frequently clouded because of predation pressure exerted on the definitive host. However, Belews Lake offers a unique opportunity for studying the population biology of *Bothriocephalus acheilognathi* because piscivorous fishes are absent from the main body of the reservoir. The reservoir was constructed in 1970, and by 1975 had developed a fish community that could be considered as typical for this section of the U.S. Within 3 yr, however, virtually the entire assemblage of fish species, including all the piscivorous fishes, had been eliminated from the main body of the reservoir because of the accumulation of lethal levels of selenium from a fly-ash settling basin (Cumbie and Van Horn, 1978).

The presence of an introduced cestode in a thermally altered aquatic ecosystem, devoid of piscivorous fishes in all but the headwaters of the lake, offered an excellent opportunity to develop and answer several questions. First, we wanted to compare the seasonal dynamics of *B. acheilognathi* in mosquitofish from ambient and thermally altered areas of the reservoir in order to evaluate the impact of artificially elevated temperatures. Second, we were interested in determining which factors are responsible for regulating the infrapopulation densities of *B. acheilognathi*. Finally, we wanted to determine if *B. acheilognathi* could be a regulatory factor in the population biology of mosquitofish; Hoffman (1980) indicated that the parasite could have significant negative effects on cyprinids grown as bait fish under hatchery conditions. The present report will focus on the first of the three questions. The other two aspects of the study will be considered elsewhere (Granath and Esch, 1983a, b).

Materials and Methods

STUDY AREA: Belews Lake (Fig. 1) is a 1,563-ha cooling reservoir located in the northern Piedmont of North Carolina. Constructed by Duke Power Company in 1970, the reservoir was designed to provide cooling water for two 1,140-megawatt turbine generators for the Belews Creek Steam Station. Cooling water is drawn from the epilimnion of the main body and subsequently discharged at 5 to 10°C above ambient temperature into the west arm of the reservoir. Water is returned to the main body of the lake via a connecting canal. Some of the intake water is used to transport bottom and fly ash from the boilers and precipitators to a 142-ha settling basin. Effluent water from the basin is returned to the reservoir via a controlled-discharge facility (Weiss and Anderson, 1978).

As alluded to earlier, the fish community developed rapidly and normally over the first 5 yr of operation. In 1975, the community consisted of 29 species (Harrell et al., 1978); currently, only five species remain in the main arm. These are mosquitofish (*Gambusia affinis*), carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), fathead minnow (*Pimephales promelas*), and red shiner (*Notropis lutrensis*). Currently, the dominant species in the reservoir are *G. affinis*, *P. promelas*, and *N. lutrensis*. The success of these species can be attributed to their obvious tolerance to selenium toxicity and the absence of piscivorous fishes.

COLLECTING PROCEDURES: Collections of *G. affinis* were made with a dip net during the first and third full weeks of each month, beginning at the end of March 1980 and continuing through the beginning of March 1982. Mosquitofish were

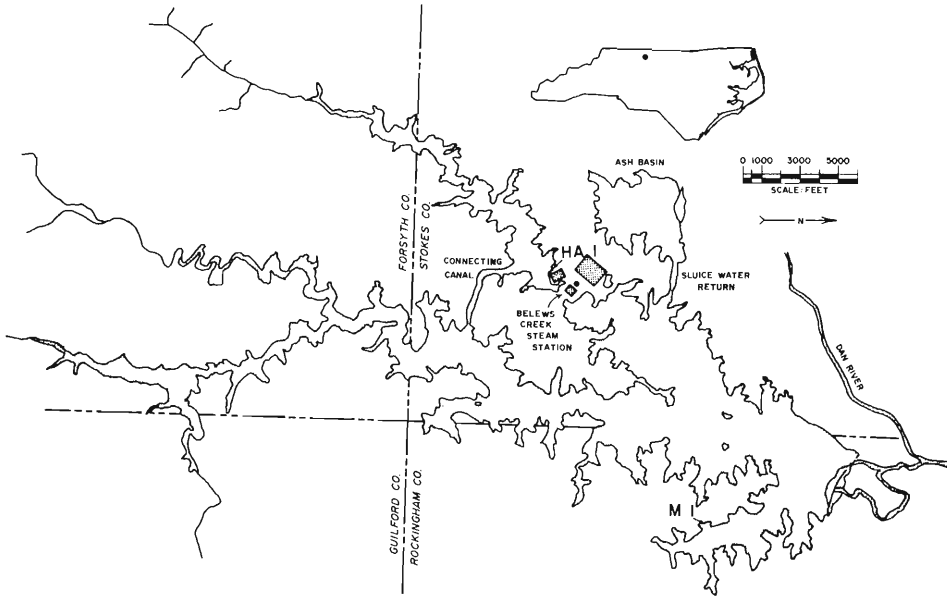


Figure 1. Map of Belews Lake showing main collecting sites in ambient (M 1) and thermally altered (HA 1) areas.

collected from the ambient (M 1) and thermally altered (HA 1) areas of the lake (Fig. 1). Each sample was comprised of 60 mosquitofish, with 20 fish in each of three arbitrarily established size classes (≤ 30 mm, 31–40 mm, and ≥ 41 mm). The fish were transported to the laboratory, where they were weighed, measured for total and standard lengths, sexed, and necropsied with the aid of a binocular dissecting microscope. During necropsy, the numbers of worms were counted.

In order to estimate the prevalence and density of *B. acheilognathi* in the natural population of mosquitofish, the following computations were made. The percentage of a randomly collected sample ($N = 60$) of fish in each of the three size classes was determined for each monthly collection (these fish represented a collection different from the fish examined for parasites). This percentage was multiplied by the mean density or prevalence of the parasite in the size class. By summing the results for all three size classes, and repeating the procedure for each monthly sample at each collecting site, an estimate of the overall prevalence and density of the parasite in the natural population of mosquitofish was determined. Estimates of prevalence and density were computed because there was no a priori indication that the entire Belews Lake mosquitofish population consisted equally of individuals in each of the three size classes, or that prevalence and density are the same in all size classes all year long.

Recruitment of *B. acheilognathi* by *G. affinis* was monitored monthly from March 1980 through February 1982. Plankton were collected monthly in the littoral zone at sites M 1 and HA 1 with several (20–30) horizontal tows of a #20-mesh Wisconsin plankton net. The plankton were returned live to the laboratory, where they were placed into 4-liter aquaria containing uninfected *G. affinis*; water temperatures were maintained at 22°C. Four days after the fish were exposed to

the plankton, four fish from each group were killed and necropsied to determine if they had acquired infections of *B. acheilognathi*. After 2 wk, the remaining fish were similarly examined.

DATA ANALYSIS: The Statistical Analysis System (SAS) (Goodnight and Barr, 1971) at TUCC (Triangle Universities Computation Center) was used for the following tests. Contingency chi-square analysis (procedure = FREQ) was performed on prevalence of the cestode among size classes and between sites. Data collected on parasite density were converted to ranks, and a one-way analysis of variance was performed (procedure = NPAR1WAY; Kruskal-Wallis test); ranks were assigned to the number of worms in each fish. Duncan's multiple range test was performed to determine whether significant differences existed among mean densities of the parasite in the three size classes of fish.

DEFINITIONS: Prevalence refers to the percentage of hosts infected with *B. acheilognathi* in a given sample. Mean density refers to the number of worms counted in a given sample, divided by the total number (both infected and uninfected) of fish within the sample. Mean intrapopulation density refers to the number of worms counted in a given sample, divided by the number of infected fish within the sample.

Results

Temperature

A normal seasonal change in surface water temperature occurred over the 2-yr study period at the ambient study site (M 1) in Belews Lake (Fig. 2). Minimum temperatures (10°C) were observed in late January–early February 1981 and 1982, and maxima (32–33°C) occurred during August 1980 and June–July 1981.

Water temperatures at the thermally altered site (HA 1) also fluctuated seasonally, but, in contrast to the ambient site, never dropped below 19°C in winter (December, January, and February) (Fig. 4). During mid- to late summer (summer includes June, July, and August) of both years, temperatures reached 40°C.

Seasonal dynamics of *B. acheilognathi* at the ambient station

Recruitment, prevalence, and density of *B. acheilognathi* in mosquitofish exhibited seasonal cycles at M 1 (Fig. 2). In both years, recruitment began in early June and continued into October. This pattern is also reflected by the almost steady increase in prevalence within all three size classes during the summer months and early fall of 1980 and 1981. Contingency chi-square analysis revealed a significant variation in prevalence among months (for ≤ 30 -mm size class in 1980, $\chi^2 = 154.0$ with 23 df, $P < 0.005$; for 31–40-mm size class in 1980, $\chi^2 = 116.8$, $P < 0.005$; for > 40 -mm size class in 1980, $\chi^2 = 88.4$, $P < 0.005$; for ≤ 30 -mm size class in 1981, $\chi^2 = 104.9$, $P < 0.005$; for 31–40-mm size class in 1981, $\chi^2 = 84.8$, $P < 0.005$; for > 40 -mm size class in 1981, $\chi^2 = 63.7$, $P < 0.005$); there was no significant difference between years at site M 1 ($\chi^2 = 62.3$, df = 71, $P > 0.05$). The same analysis also indicated that prevalence among size classes varied significantly only during March and April in 1980.

Mean densities of *B. acheilognathi* changed in a manner similar to seasonal changes in prevalence (Fig. 2). Lowest densities occurred in the summer months of both years in all three size classes of mosquitofish, with peaks in early winter.

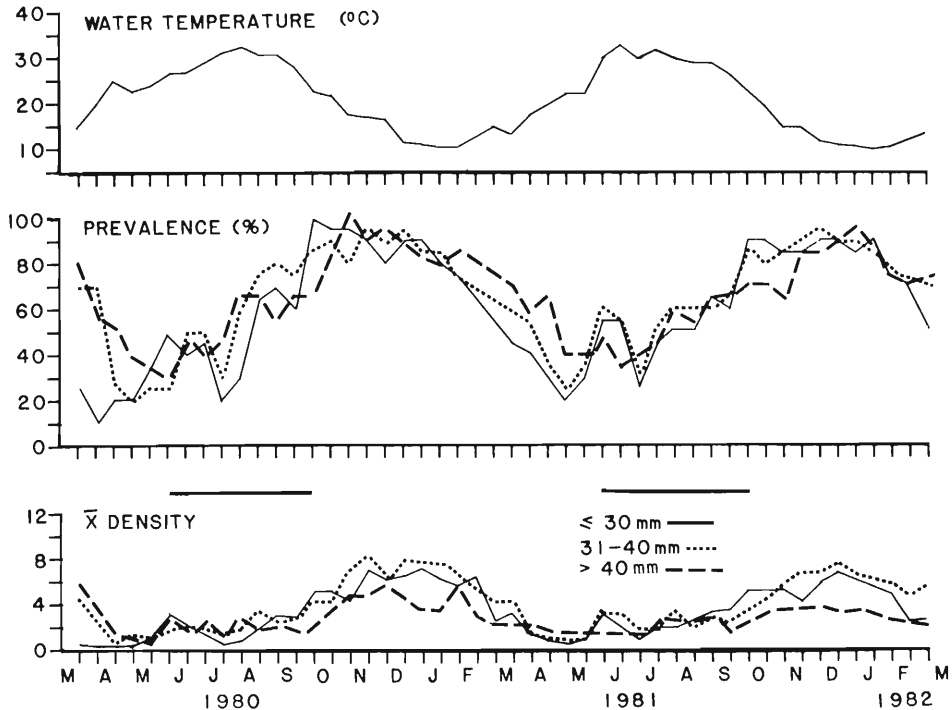


Figure 2. Bi-weekly changes in temperature, and prevalence and density of *Bothriocephalus acheilognathi* within three size classes of *Gambusia affinis* from the ambient site (M 1). The horizontal bar indicates when recruitment of the cestode occurred.

Analysis of variance revealed a significant seasonal change in mean density and a difference in density among the size classes of *G. affinis*, although there was no difference between the two years of study ($F = 0.98$, $df = 45, 2,791$, $P > 0.05$). Duncan's multiple range test indicated that the largest size class of mosquitofish (> 40 mm) had a lower density of *B. acheilognathi* than the other two size classes, although some deviation from this trend was apparent (Tables 1, 2). Mean densities were consistently lower in larger size classes during the winter months of both years.

The prevalence and density of *B. acheilognathi* were estimated for the entire mosquitofish population (Fig. 3). The estimates indicate that prevalence increased steadily during late summer and fall. Lowest prevalence occurred from late spring to early summer during both years of study. Estimated mean densities of *B. acheilognathi* varied in a pattern similar to that of estimated prevalence (Fig. 3). Thus, lowest densities were in summer, with peaks in early winter.

Seasonal dynamics of *B. acheilognathi* at the thermally altered site

The population dynamics of *B. acheilognathi* varied seasonally at the thermally altered site (Fig. 4). Recruitment of the cestode began in May of both years and continued through October of 1980 and November of 1981; it stopped, however, in August of each year. In 1980, prevalence peaked in all size classes of mosqui-

Table 1. Mean density of *Bothriocephalus acheilognathi* from three size classes of mosquitofish collected from the ambient site (M 1) from March 1980 to March 1981. (Each mean is based on *N* = 20.)

Date	Size class (mm) ($\bar{x} \pm \text{SE}$)†			<i>F</i> ‡ (df = 2,57)
	≤30	31–40	≥41	
3/80	0.4 ± 0.2	4.6 ± 1.3	6.0 ± 1.7	8.61*
4/80	0.2 ± 0.1	2.4 ± 0.6	4.0 ± 1.2	6.33*
4/80	0.3 ± 0.2	0.8 ± 0.3	1.6 ± 0.8	1.25 ^{ns}
5/80	0.4 ± 0.2	1.4 ± 0.9	1.1 ± 0.4	1.42 ^{ns}
5/80	1.1 ± 0.4	0.4 ± 0.2	1.1 ± 0.7	2.60 ^{ns}
6/80	3.6 ± 1.1	1.5 ± 0.9	2.4 ± 1.8	5.91*
6/80	2.4 ± 1.7	2.3 ± 1.0	1.5 ± 0.5	6.31*
7/80	1.4 ± 0.5	1.9 ± 0.7	2.3 ± 0.9	5.11 ^{ns}
7/80	0.2 ± 0.1	1.6 ± 0.9	1.1 ± 0.4	3.12 ^{ns}
8/80	0.7 ± 0.3	1.8 ± 0.4	2.4 ± 0.8	5.50 ^{ns}
8/80	2.0 ± 0.6	3.4 ± 1.2	2.0 ± 0.5	1.83 ^{ns}
9/80	3.0 ± 0.9	2.5 ± 0.6	2.4 ± 1.2	2.26 ^{ns}
9/80	2.7 ± 0.7	2.8 ± 0.8	1.7 ± 0.5	4.97 ^{ns}
10/80	5.0 ± 1.0	4.1 ± 0.9	1.8 ± 0.4	8.96*
10/80	5.3 ± 1.2	4.3 ± 0.8	3.1 ± 0.7	7.43*
11/80	4.2 ± 0.7	7.0 ± 1.8	4.7 ± 0.7	8.32*
11/80	7.0 ± 1.6	8.1 ± 1.3	4.8 ± 1.2	9.69*
12/80	6.1 ± 1.5	5.7 ± 1.0	5.7 ± 0.8	2.49 ^{ns}
12/80	6.5 ± 1.0	8.0 ± 1.2	4.9 ± 0.7	5.60 ^{ns}
1/81	7.8 ± 1.4	7.6 ± 1.2	3.9 ± 0.5	11.40*
1/81	6.2 ± 1.7	7.4 ± 1.7	3.6 ± 0.6	12.15*
2/81	5.6 ± 1.3	6.5 ± 1.1	4.8 ± 1.0	3.63 ^{ns}
2/81	6.4 ± 1.7	5.5 ± 1.7	3.3 ± 0.7	7.13*
3/81	2.5 ± 0.8	4.5 ± 2.0	2.4 ± 0.6	5.03 ^{ns}
<i>F</i> (df = 23,456)	11.42*	8.39*	6.30*	

† Underscored means are not significantly different as determined by Duncan's multiple range test.
‡ * = significant at *P* < 0.005. ^{ns} = not significant at *P* > 0.005.

tofish during June and early July, before declining to the lowest annual levels in mid- to late summer. Prevalence then increased through late fall and remained relatively high throughout the winter in fish ≤30 mm in total length. Within the smallest size class, highest prevalence was noted in November and then declined steadily until May. The sharp increases in prevalence noted in the summer of 1980 were not observed in 1981. Contingency chi-square analyses indicated that there were significant variation in prevalence of *B. acheilognathi* among collection periods in each size class of fish during each year of the study (for ≤30-mm size class in 1980, $\chi^2 = 112.6$ with 23 df, *P* < 0.005; for 31–40 mm in 1980, $\chi^2 = 126.6$, *P* < 0.005; for >40 mm in 1980, $\chi^2 = 119.5$, *P* < 0.005; for ≤30 mm in 1981, $\chi^2 = 127.3$, *P* < 0.005; for 31–40 mm in 1981, $\chi^2 = 146.1$, *P* < 0.005; for >40 mm in 1981, $\chi^2 = 155.5$, *P* < 0.005). There was, however, no significant

Table 2. Mean density of *Bothriocephalus acheilognathi* from three size classes of mosquitofish collected from the ambient site (M 1) from March 1981 to March 1982. (Each mean is based on *N* = 20.)

Date	Size class (mm) ($\bar{x} \pm \text{SE}$)†			<i>F</i> ‡ (df = 2,57)
	≤30	31–40	≥41	
3/81	3.1 ± 0.9	4.4 ± 1.3	2.3 ± 0.4	5.51 ^{ns}
4/81	1.3 ± 0.4	1.4 ± 0.4	2.2 ± 0.5	2.10 ^{ns}
4/81	0.5 ± 0.2	0.9 ± 0.3	1.7 ± 0.7	1.56 ^{ns}
5/81	0.4 ± 0.2	0.8 ± 0.3	1.2 ± 0.4	1.89 ^{ns}
5/81	0.6 ± 0.4	0.7 ± 0.2	1.1 ± 0.4	1.65 ^{ns}
6/81	3.0 ± 0.7	3.9 ± 1.1	1.2 ± 0.4	6.39*
6/81	2.0 ± 0.8	3.2 ± 0.8	1.5 ± 0.5	6.87*
7/81	0.7 ± 0.3	1.8 ± 0.9	1.5 ± 0.5	4.15 ^{ns}
7/81	1.8 ± 0.5	3.7 ± 2.1	2.2 ± 0.7	5.11 ^{ns}
8/81	1.9 ± 0.7	3.7 ± 1.0	2.5 ± 0.7	7.26*
8/81	2.4 ± 0.9	2.0 ± 0.5	2.3 ± 0.7	0.11 ^{ns}
9/81	3.3 ± 0.8	2.8 ± 0.7	2.8 ± 0.8	1.17 ^{ns}
9/81	3.3 ± 0.8	2.5 ± 0.6	2.2 ± 0.5	2.23 ^{ns}
10/81	5.1 ± 0.8	3.3 ± 0.8	2.3 ± 0.4	6.98*
10/81	5.3 ± 0.9	4.3 ± 1.0	3.1 ± 0.8	8.19*
11/81	5.1 ± 1.2	5.6 ± 1.2	3.2 ± 0.8	8.06*
11/81	4.2 ± 1.2	6.6 ± 1.2	3.6 ± 0.7	9.11*
12/81	5.9 ± 1.4	6.7 ± 1.5	3.8 ± 0.8	5.99*
12/81	6.6 ± 1.4	7.1 ± 1.9	3.4 ± 0.7	8.32*
1/82	6.1 ± 1.3	6.4 ± 1.3	3.3 ± 0.6	8.79*
1/82	5.6 ± 1.0	6.3 ± 1.1	3.0 ± 0.6	9.06*
2/82	5.1 ± 1.5	6.2 ± 1.5	3.1 ± 0.9	10.17*
2/82	2.5 ± 0.6	4.6 ± 1.1	2.9 ± 0.6	5.06 ^{ns}
3/82	2.6 ± 0.9	6.0 ± 1.8	2.3 ± 0.6	10.10*
<i>F</i> (df = 23,456)	6.91*	5.13*	2.61*	

† Underscored means are not significantly different as determined by Duncan's multiple range test.
‡ * = significant at *P* < 0.005. ^{ns} = not significant at *P* > 0.005.

difference between years ($\chi^2 = 55.2$, df = 71, *P* > 0.05). Examination of the data in Figure 4 clearly shows that prevalence of *B. acheilognathi* was consistently lowest in the smallest size class of fish during the winter and spring months of both years.

Changes in mean densities of the cestode followed a seasonal pattern in both years of the study (Fig. 4). Highest densities were recorded in fall and early winter and lowest levels were reached in mid- to late summer. Analysis of variance revealed a significant seasonal change in mean density, but no difference between the two years of study (*F* = 0.93, df = 47,2,771, *P* > 0.05). Duncan's multiple range test indicated that, in general, the small size class had fewer parasites in late summer and winter, whereas the largest size class (>40 mm) had the lowest densities during other months of the year (Tables 3, 4).

The estimated seasonal prevalences and mean densities of infection at the

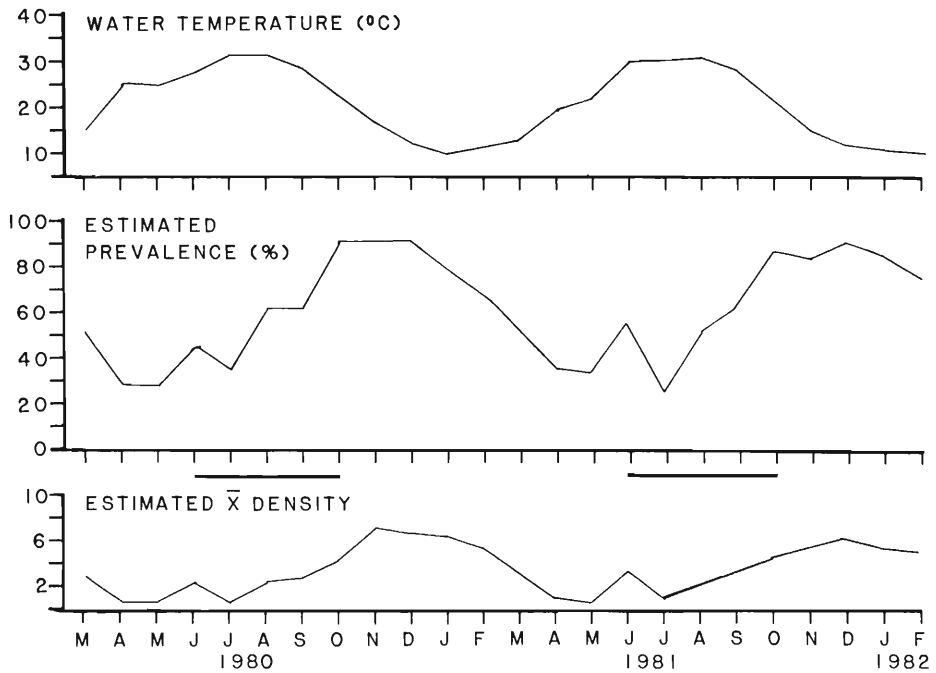


Figure 3. Monthly changes in temperature, and estimated prevalence and density of *Bothriocephalus acheilognathi* within *Gambusia affinis* from the ambient site (M 1). The horizontal bar indicates when recruitment of the cestode occurred.

thermally altered site are given in Figure 5. The estimates suggest a spring increase in prevalence during the first year, but not in the second. Lowest prevalences were observed in the summer of both years, with rises during the fall of both years. Estimated changes in densities of *B. acheilognathi* appeared to follow the pattern seen for prevalences. Thus, lowest densities occurred in the summer of both years, with a rise in the spring of the first year, but not the second.

Discussion

Seasonal changes in the dynamics of most helminth parasites in aquatic ecosystems may be affected by a wide range of factors, of both biotic and abiotic origin (Chubb, 1980). Perhaps the most significant are normal temperature variations (Aho et al., 1982; Camp et al., 1982; reviewed by Esch, 1983) and factors associated with host reproductive and foraging strategies (Smith, 1973; Anderson, 1976; reviewed by Esch, 1983).

In both ambient and thermal locations, lowest densities and prevalence of *B. acheilognathi* were observed when water temperatures were highest, whereas peak densities and prevalences were recorded as water temperatures declined, especially during late fall. Reproduction by *G. affinis* in the ambient areas of Belews Lake was seasonal, beginning in April and continuing through October of both years. In the heated location, eyed embryos (indicating reproductive activity) were present in all months of the year. Because of continuous host reproductive activity in both areas during the summer months, some of the decline in density and

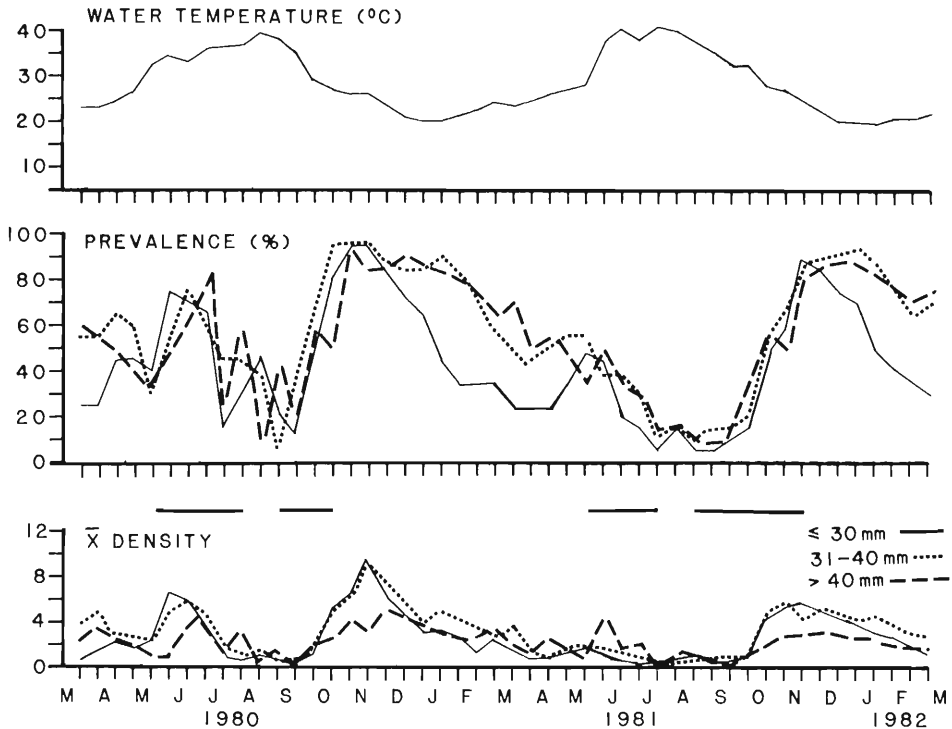


Figure 4. Bi-weekly changes in temperature, and prevalence and density of *Bothriocephalus acheilognathi* within three size classes of *Gambusia affinis* from the thermally altered site (HA 1). The horizontal bar indicates when recruitment occurred.

prevalence of the cestode at higher temperatures may be attributed to a dilution effect created by the addition of uninfected individuals into an expanding host population. Because of continuous reproductive activity by *G. affinis* during the winter at the heated station and the absence of parasite recruitment, the dilution effect is especially obvious in smaller size classes of fish.

The patterns of parasite recruitment by *G. affinis* in ambient and thermally altered areas of Belews Lake are somewhat similar to those observed by Aho et al. (1982) for *Diplostomulum scheuringi* in Par Pond, a South Carolina cooling reservoir. In the heated locations of Par Pond, recruitment of *D. scheuringi* by *G. affinis* began earlier, lasted longer, and was interrupted in the summer for a period of several weeks when water temperatures rose above 40°C. The same pattern of recruitment was observed for *B. acheilognathi* in Belews Lake. The interruption in Belews Lake, however, corresponded with a period of sharp decline in prevalence and density of *B. acheilognathi*, especially in the heated location. There was also massive mortality among *G. affinis* at the heated station during the same time, which would contribute to decreases in density and prevalence. Mortality of mosquitofish in the ambient areas of Belews Lake was not observed. Other factors, presumably of a density-dependent nature, are believed to be involved in keeping densities low during the period when active recruitment is taking place (Granath and Esch, 1983a, b).

Table 3. Mean density of *Bothriocephalus acheilognathi* from three size classes of mosquitofish collected from the thermally altered site (HA 1) from March 1980 to March 1981. (Each mean is based on $N = 20$ unless otherwise noted.)

Date	Size class (mm) ($\bar{x} \pm SE$)†			F^{\ddagger} (df = 2,57)
	≤30	31–40	≥41	
3/80	0.5 ± 0.2	4.0 ± 1.5	2.2 ± 0.7	8.13*
4/80	1.4 ± 0.7	5.7 ± 1.9	3.6 ± 1.8	10.12*
4/80	2.1 ± 0.8	2.9 ± 1.1	2.2 ± 0.7	0.16 ^{ns}
5/80	1.7 ± 0.7	2.8 ± 0.9	2.1 ± 0.7	1.11 ^{ns}
5/80	2.2 ± 0.8	2.1 ± 1.4	0.8 ± 0.4	3.29 ^{ns}
6/80	6.6 ± 1.6	4.7 ± 1.8	1.0 ± 0.3	13.62*
6/80	5.9 ± 1.9	5.8 ± 2.0	3.8 ± 1.3	8.29*
7/80	4.0 ± 1.4	5.0 ± 1.5	4.3 ± 0.5	1.56 ^{ns}
7/80	1.0 ± 0.7	2.0 ± 0.8	1.4 ± 0.8	1.98 ^{ns}
8/80	0.5 ± 0.2	1.2 ± 0.4	4.0 ± 1.2	12.01*
8/80	1.0 ± 0.3	1.1 ± 0.3	§0.2 ± 0.2	2.06 ^{ns}
9/80	0.5 ± 0.3	0.1 ± 0.1	1.2 ± 0.6	3.16 ^{ns}
9/80	0.4 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.05 ^{ns}
10/80	0.9 ± 0.3	1.9 ± 0.5	1.4 ± 0.4	1.09 ^{ns}
10/80	5.2 ± 1.2	4.9 ± 1.2	3.1 ± 0.7	5.31 ^{ns}
11/80	6.3 ± 1.3	6.0 ± 0.9	5.1 ± 0.7	4.53 ^{ns}
11/80	9.6 ± 1.7	9.2 ± 1.6	3.1 ± 0.6	17.16*
12/80	5.7 ± 1.4	7.8 ± 1.9	5.0 ± 0.8	5.05 ^{ns}
12/80	4.5 ± 1.3	5.3 ± 1.2	4.4 ± 0.9	4.96 ^{ns}
1/81	3.1 ± 0.7	3.9 ± 0.9	3.9 ± 1.0	1.03 ^{ns}
1/81	3.5 ± 1.3	4.7 ± 0.8	3.0 ± 0.5	1.96 ^{ns}
2/81	2.5 ± 1.1	4.1 ± 0.9	2.1 ± 0.4	4.89 ^{ns}
2/81	1.2 ± 0.4	3.5 ± 0.6	2.1 ± 0.6	5.12 ^{ns}
3/81	2.3 ± 0.9	2.7 ± 0.9	3.0 ± 0.8	0.96 ^{ns}
$F(df)$	7.82* (23,456)	7.03* (23,456)	5.61* (23,421)	

† Underscored means are not significantly different as determined by Duncan's multiple range test.

‡ * = significant at $P < 0.005$, ^{ns} = not significant at $P > 0.005$.

§ Entry based on $N = 6$, $df = 2,43$.

Normal changes in host foraging strategies with increasing age and/or size may also affect the population biology of helminth parasites (Kennedy, 1977; Chubb, 1980; Esch, 1983) and appear to play a significant role for the mosquitofish–*B. acheilognathi* system in Belews Lake. A recently completed, but as yet unpublished, study of the stomach contents of mosquitofish in Belews Lake strongly suggests definite changes in foraging and prey preference within and between various size classes of *G. affinis* throughout the year (M. R. Riggs, pers. comm.). During those months when water temperatures were high (late spring through fall), stomachs were full, reflecting higher feeding intensity or, perhaps, greater prey availability. This time period corresponds to those parts of the year when recruitment of *B. acheilognathi* was occurring.

Table 4. Mean density of *Bothriocephalus acheilognathi* from three size classes of mosquitofish collected from the thermally altered site (HA 1) from March 1981 to March 1982. (Each mean is based on $N = 20$ unless otherwise noted.)

Date	Size class (mm) ($\bar{x} \pm SE$)†			F_{\dagger} (df = 2,57)
	≤ 30	31–40	≥ 41	
3/81	1.3 \pm 0.6	3.6 \pm 0.9	1.8 \pm 0.4	6.10*
4/81	0.5 \pm 0.2	1.6 \pm 0.6	1.5 \pm 0.5	5.21 ^{ns}
4/81	0.7 \pm 0.4	0.9 \pm 0.3	2.5 \pm 0.6	6.17*
5/81	1.0 \pm 0.3	1.9 \pm 0.5	1.8 \pm 0.5	0.98 ^{ns}
5/81	1.8 \pm 0.5	2.1 \pm 0.5	1.1 \pm 0.4	1.17 ^{ns}
6/81	1.0 \pm 0.3	1.5 \pm 0.6	4.1 \pm 1.2	7.51*
6/81	0.4 \pm 0.2	1.1 \pm 0.4	1.7 \pm 1.3	5.03 ^{ns}
7/81	0.2 \pm 0.1	0.8 \pm 0.3	2.0 \pm 1.0	5.12 ^{ns}
7/81	0.3 \pm 0.3	0.3 \pm 0.2	0.2 \pm 0.1	0.08 ^{ns}
8/81	0.6 \pm 0.4	0.5 \pm 0.3	0.6 \pm 0.5	0.03 ^{ns}
8/81	0.8 \pm 0.7	0.3 \pm 0.2	0.2 \pm 0.1	0.16 ^{ns}
9/81	0.3 \pm 0.3	1.0 \pm 0.6	0.6 \pm 0.4	1.01 ^{ns}
9/81	0.4 \pm 0.3	0.7 \pm 0.4	0.4 \pm 0.2	1.00 ^{ns}
10/81	0.6 \pm 0.4	1.0 \pm 0.5	0.6 \pm 0.3	1.12 ^{ns}
10/81	4.3 \pm 1.3	4.3 \pm 1.0	2.1 \pm 0.5	3.68 ^{ns}
11/81	5.2 \pm 1.0	5.7 \pm 1.9	2.9 \pm 1.1	7.38*
11/81	5.5 \pm 1.0	4.2 \pm 0.9	2.8 \pm 0.5	8.42*
12/81	4.8 \pm 1.6	5.2 \pm 1.3	3.4 \pm 0.7	5.96*
12/81	4.4 \pm 1.0	5.0 \pm 1.6	2.7 \pm 0.4	9.01*
1/82	3.5 \pm 1.0	4.2 \pm 1.1	2.4 \pm 0.4	5.24 ^{ns}
1/82	3.2 \pm 1.1	4.5 \pm 1.1	2.8 \pm 0.6	5.41 ^{ns}
2/82	2.9 \pm 1.0	4.1 \pm 0.7	2.4 \pm 0.5	6.12*
2/82	2.1 \pm 0.8	2.8 \pm 0.7	2.1 \pm 0.5	1.05 ^{ns}
3/82	1.5 \pm 0.8	2.6 \pm 0.7	1.8 \pm 0.4	2.13 ^{ns}
$F(df)$	7.97* (23,456)	7.64* (23,456)	6.04* (23,441)	

† Underscored means are not significantly different as determined by Duncan's multiple range test.

‡ * = significant at $P < 0.005$. ^{ns} = not significant at $P > 0.005$.

§ Entry based on $N = 6$, $df = 2,43$.

It was initially assumed that prey preference of mosquitofish was similar among all size classes, but this assumption was erroneous. Riggs (pers. comm.), in his study on the diet of *G. affinis* in Belews Lake, found that large fish (>40 mm) fed primarily on dipterans and rarely on copepods. Smaller fish fed mainly on small insects and copepods. Similar changes in the diet of fish with increasing body size have been observed for other species as well (Werner, 1974; Keast, 1980). Lowest densities of the cestode were most commonly seen in the largest size class, and thus may be related to variation in dietary preference as the host increases in size.

Seasonal changes in the diet of mosquitofish were also noted by Riggs (pers. comm.). The two smaller groups (≤ 40 mm) consumed insect larvae and copepods during the warmer months and only insects during the cooler months. Larger

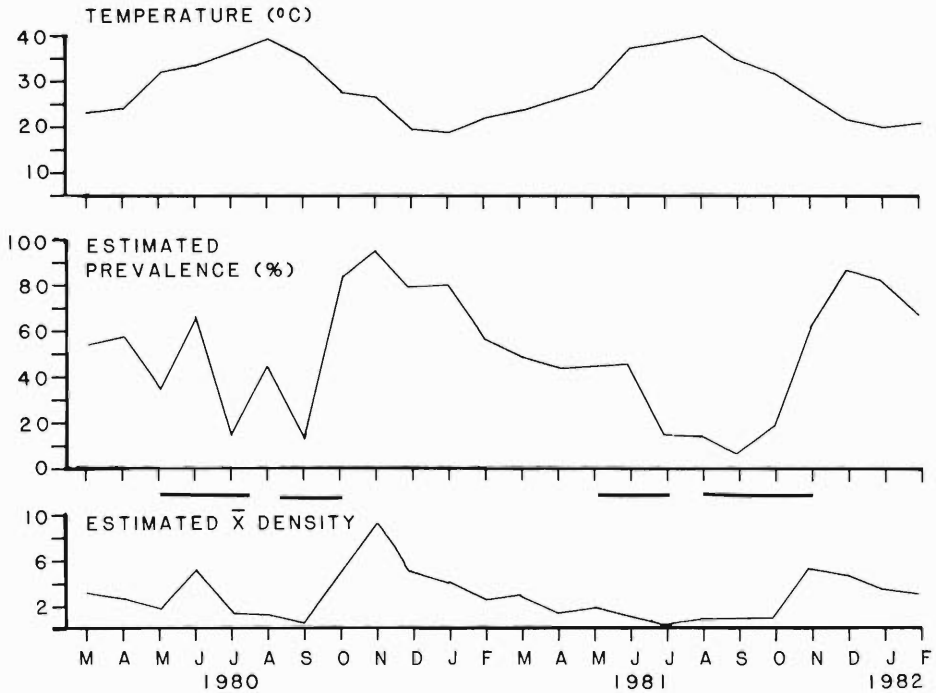


Figure 5. Monthly changes in temperature, and estimated prevalence and density of *Bothriocephalus acheilognathi* within *Gambusia affinis* from the thermally altered site (HA 1). The horizontal bar indicates when recruitment occurred.

mosquitofish (>40 mm) fed primarily on large insects and their larvae during most of the year, although some copepods were consumed during late summer and fall. Similar changes in prey consumption by other fish species have been noted previously (Ball and Kilambi, 1973; Smith, 1973).

The variable foraging patterns of *G. affinis* may partially explain the seasonal fluctuations in density and prevalence of *B. acheilognathi*. Densities of the cestode in the larger size class were generally lower than in other size classes throughout the year. Because the former rarely fed on copepods, recruitment probability for the parasite would be reduced. On the other hand, prevalences of the parasite were similar among all three size classes, increasing during late summer and fall. This period coincides with the only time during which copepods formed a substantial portion of the diet among larger mosquitofish. All parasite recruitment ceased in late fall and did not resume until the following spring. Although reproductive activity of the fish was continuous at the heated site throughout the year and could be important (as a dilution factor) in understanding changes in density and prevalence during the cooler months, reproductive activity was not continuous in ambient areas, yet prevalence and density still declined. Such changes may be the result of density-dependent factors, and will be discussed elsewhere (Granath and Esch, 1983a).

Another factor that could be of significance in influencing the dynamics of *B. acheilognathi* in Belews Lake is the seasonal change in abundance of copepod

intermediate hosts. Although not the focus of the present study, other investigators have examined seasonal changes in Belews Lake plankton. Anderson and Lenat (1978) and Weiss and Anderson (1978) reported that copepod densities rose in spring, peaked in mid-summer, and declined to lowest levels by late fall. Thus, highest numbers of copepods were present during periods of warmest water temperatures and when active recruitment of *B. acheilognathi* by *G. affinis* could be demonstrated experimentally. A similar pattern in seasonal dynamics of *Bothriocephalus rarus* was noted by Jarroll (1979). He observed peak densities of *Mesocyclops ater* at a time when active recruitment by the definitive host was also occurring.

The spatial distributions of copepod intermediate hosts and *G. affinis* were also considered as potential factors in affecting the population biology of *B. acheilognathi*. *Mesocyclops edax* and *Cyclops bicuspidatus thomasi* are limnetic in distribution, and *Tropocyclops prasinus* is both littoral and limnetic (Pennak, 1966). Normally, mosquitofish are restricted to the shallow portions of the littoral zone, using vegetation for lateral concealment (Casterlin and Reynolds, 1977). Thus, *G. affinis* would normally be forced to prey primarily on *T. prasinus*. However, the absence of piscivorous fishes has permitted *G. affinis* to expand its lateral spatial distribution significantly in Belews Lake. Indeed, mosquitofish have been collected up to 300 m from shore, at the surface of water 40 m deep. Furthermore, uninfected mosquitofish fed plankton that had been collected 300 m from shore became infected with *B. acheilognathi*. This observation strongly suggests that transmission of *B. acheilognathi* is not confined to the littoral zone and that the spatial distribution of copepod intermediate hosts is not a factor affecting the population biology of the cestode in Belews Lake.

Acknowledgments

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Purification of the Major Concanavalin A-Binding Surface Glycoprotein from Adult *Schistosoma mansoni*¹

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ABSTRACT: Radiolabeled freeze-thaw and detergent-extracted antigens from adult *Schistosoma mansoni* were fractionated by concanavalin A-Sepharose 4B affinity chromatography to isolate specific surface membrane glycoproteins. SDS-PAGE and ampholine isoelectric focusing of the α -methylmannoside eluate reveal the major component to have a molecular weight of approximately 58,000 and pI value of approximately 4.8. Surface localization of the glycoprotein is inferred by its extraction by low concentrations of non-ionic detergent, and by the ability of fluorescein-labeled Con A to bind to the surface of adult worms but not internally. Surface binding of Con A is inhibited by the purified glycoprotein. The glycoprotein reacts with sera from infected humans, as shown by enzyme-linked immunoassay (EIA). The protocol for large-scale purification of this antigen is described in detail. An ancillary result of this work is the isolation of a second glycoprotein, not necessarily a surface antigen, that has a molecular weight of approximately 300,000, pI value of approximately 4.2, and also reacts with sera from infected humans.

Numerous reports have documented the presence of parasite antigens that stimulate a wide spectrum of immune effector mechanisms involving antibodies, complement fixation, and host cells (see review by Ogilvie et al., 1980). Although the relevance of these reactions to protective immunity is not well established, there can be little doubt that the detection, extraction, and isolation of antigens is central to any understanding of their role in immunity.

The presence of surface antigens that bind concanavalin A has been demonstrated for both *Schistosoma mansoni* schistosomula (Murrell et al., 1978) and adult worms (Simpson and Smithers, 1980). By using Con A affinity chromatography, Bennett and Seed (1977) isolated a glycoprotein fraction from adult *S. mansoni* consisting of picogram quantities of several components. However, efforts to "scale up" production have been hampered by the heterogeneity of tegumental antigen preparations, nonspecific (and sometimes irreversible) binding of material to Con A-Sepharose columns, and low yields of the final product. By using a sequence of column separations, we have been able to isolate microgram amounts of a Con A-binding surface glycoprotein from adult *S. mansoni*. The purified antigen migrates as a single band on SDS gels.

Materials and Methods

Collection of parasites

Adult worms of a Puerto Rican strain of *S. mansoni* were obtained from anesthetized NIH/NMRI mice 7 wk after infection, by portal perfusion with citrated saline. Worms were collected on a 183- μ m-mesh Nitex screen (Tetko Inc., New York) and washed several times with citrated saline; pieces of debris and visibly

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damaged worms were removed by pipette. In addition to live material, lyophilized *S. mansoni* adult worms were obtained in 100-mg (dry weight) aliquots from the WHO Special Programme for Research and Training in Tropical Diseases.

Crude antigen preparation

A freeze-thaw preparation of adult worms (AFT-1) was made as described previously (Murrell et al., 1974). Briefly, adult worms were frozen in Hanks' balanced salt solution, pH 7.2, at -70°C . After thawing at room temperature, the mixture was filtered through a Nitex screen to remove worm carcasses and the filtrate centrifuged at 10,000 *g* for 30 min; the supernatant was then dialyzed against 0.15 M borate-buffered saline (BBS), pH 7.6, and concentrated by Amicon® PM-10 membrane filtration (10 μm) to a final concentration of 5–8 mg/ml.

A detergent extract was prepared by incubating approximately 1,000 worms in 5 ml borate-buffered saline (BBS) containing 0.5% aprotinin enzyme inhibitor (Trasylol®, FBA Pharmaceuticals, New York) and 0.05% Nonidet P-40 for 15 min at 37°C . The mixture was centrifuged at 1,000 *g* for 5 min and the supernatant collected.

Lipids were extracted from the lyophilized worms with chloroform : methanol (following Hayunga et al., 1982). Approximately 50 mg (dry weight) of lyophilized *S. mansoni* was suspended in 5 ml chloroform : methanol (2:1) and disrupted in a ground-glass tissue homogenizer. After 2 hr extraction at room temperature, the mixture was centrifuged at 3,000 *g* for 30 min and the supernatant removed; a second extraction was done overnight at 4°C . The pellet was next resuspended in 1 ml of 0.5% Nonidet P-40 in BBS, incubated for 30 min at 37°C , and centrifuged at 3,000 *g* for 30 min. The supernatant was dialyzed against several changes of BBS.

Protein radioiodination

Protein labeling with ^{125}I -iodinated *p*-hydroxyphenylpropionic acid, N-hydroxysuccinimide ester, was carried out following the method originally described by Bolton and Hunter (1973). Approximately 100 μg protein in a 300- μl volume was radioiodinated with 0.5 mCi Bolton-Hunter reagent (specific activity 4,000 Ci/mmol, molecular weight 511.2) (New England Nuclear) in each experiment. The detergent extract was dialyzed against 0.1 M borate buffer, pH 8.5, prior to labeling; the more concentrated freeze-thaw antigen was simply diluted 1:10 in the buffer. After labeling for 30 min at 4°C , the reaction was terminated by the addition of 500 μl 1% glycine in borate buffer. Samples were then dialyzed against 1-liter volumes of 0.1 M sodium acetate buffer (pH 6.8, containing 0.5 mM Ca^{++} and Mn^{++}) to remove unbound label and to facilitate the next step in the fractionation procedure. Dialysis was considered complete when the buffer contained less than 2,000 cpm/ml and when at least 75% of the labeled sample could be precipitated by 20% w/v trichloroacetic acid (TCA); four or five changes of buffer were usually sufficient.

Affinity chromatography

Analytical separation using concanavalin A or *Lens culinaris* (lentil) lectin was performed as described previously (Hayunga et al., 1982). After exhaustive dialysis

against the 0.1 M sodium acetate buffer, an aliquot (=approximately 1,000,000 cpm) of labeled antigen was applied to a 0.5×10 -cm column of concanavalin A-Sepharose 4B (Pharmacia) or lentil lectin-Sepharose 4B (Pharmacia) at a flow rate of 0.2 ml/min. After collection of the initial peak, the column was thoroughly washed with 300 ml acetate buffer, then eluted with acetate buffer containing 0.1 M α -methylmannoside (methyl- α -D-mannopyranoside). The eluate was dialyzed against distilled water, then fractionated by ampholine isoelectric focusing column, or lyophilized and reconstituted in sample buffer for SDS-PAGE, as described below.

Preparative-scale affinity chromatography was performed using a 0.9×20 -cm concanavalin A-Sepharose 4B column equilibrated with acetate buffer, at a flow rate of 0.2 ml/min. Initially, samples consisted of 1–2 ml (approximately 5–10 mg) AFT-1 antigen that had been dialyzed against acetate buffer. Bound material was eluted from the column using acetate buffer containing 0.1 M α -methylmannoside, followed by 0.15 M BBS (pH 7.6), then 0.45 M borate buffer (pH 6.0). Later separations were done by application of 15–20 ml (approximately 3 mg) of eluate from a Sephacryl S-200 column (described below), thorough washing with approximately 500 ml acetate buffer, then elution using 0.45 M borate. In all cases, the eluted fractions were pooled and dialyzed against distilled water for subsequent analysis.

Gel filtration

Aliquots of 5 mg of lipid-extracted antigen (1 mg/ml protein) in 0.1 M acetate buffer, pH 6.8, were applied to a 1.5×25 -cm column of Sephacryl S-200 Superfine (Pharmacia). The column was eluted with acetate buffer at a flow rate of 3 ml/hr and the eluate was collected in 2-ml fractions. Absorbance at 280 nm was determined using a Gilford Model 250 spectrophotometer.

SDS-PAGE

Lyophilized samples were resuspended in 150–200 μ l sample buffer consisting of 0.04 M Tris-HCl (pH 7.0) containing 2% SDS, 20% glycerol, 50 mM dithiothreitol (DTT), and 20 μ g/ml bromophenol blue. Electrophoresis was performed using 5-mm-i.d. tubes in a continuous buffer system at 2.5 mA per tube for 2.5 hr. The electrophoresis buffer consisted of 14.4 g/liter glycine, 3 g/liter Tris, and 1 g/liter SDS, pH 8.0. The 7½% running gel consisted of 76.4 g/liter acrylamide, 2.01 g/liter N,N'-methylenebisacrylamide, 0.52 g/liter SDS, 0.3 ml/liter TEMED, and 0.37 g/liter ammonium persulfate in 0.4 M Tris-HCl, pH 7.0; total volume was 2.5 ml per tube. The stacking gel consisted of 30 g/liter acrylamide, 0.79 g/liter N,N'-methylenebisacrylamide, 1.43 g/liter SDS, 0.36 ml/liter TEMED, and 0.8 g/liter ammonium persulfate in 0.14 M Tris-HCl, pH 7.0; total volume was 200 μ l per tube. Gels were removed from the glass tubes, frozen, cut into 2-mm slices using a Bio-Rad gel slicer, and counted using an LKB 1270 RackGamma II.

Isoelectric focusing

After dialysis against distilled water (to remove salts), samples were applied to a 110-ml LKB Multiphor® ampholine isoelectric focusing column and run for 18 hr at 15 W, maximum voltage 1,600 V. A discontinuous sucrose gradient was

made by manual layering of sucrose solutions containing ampholine. Sample was added to the middle of the gradient. Separations were performed using pH range of 3.5–10.0 or 2.5–6.0. Detailed description of ampholine mixtures and sample application may be found in literature supplied by the manufacturer.

Reactivity with antisera

The ability of the purified antigen to react with antisera was evaluated by enzyme-linked immunoassay (EIA) as described by Stek and Kassim (1983). Briefly, 0.2–1.0 μg of antigen was used to coat the plastic wells of microtiter plates, then was incubated with antisera followed by alkaline phosphatase-labeled goat anti-human IgG and *p*-nitrophenyl phosphate substrate in a “sandwich” fashion. Sera from patients infected with either *S. mansoni* or *Schistosoma haematobium* were obtained from Dr. E. Higashi, NAMRU-3, Cairo; *Schistosoma japonicum*, from Dr. J. Cross, NAMRU-2, Manila.

Inhibition of antibody binding

Approximately 0.4 μg of antigen was used to coat the plastic wells of microtiter plates, as in a typical EIA. The next incubation, however, consisted of specific antibody (human *S. mansoni*-infection serum) diluted 1:100 in solutions of various monosaccharides. Thus, if the monosaccharide were to compete with structurally similar binding sites of the antigen, antibody binding and subsequent colorimetric reaction would be reduced. Inhibition was measured using nine sugars and amino sugars (D-galactose [gal], D-galactosamine [galNH], N-acetyl-D-galactosamine [galNAc], D-glucose [glc], D-glucosamine [glcNH], N-acetyl-D-glucosamine [glcNAc], D-mannose [man], D-fucose [fuc], and methyl- α -D-mannopyranoside [Me- α -man]) prepared in concentrations of 1, 10, 100, and 1,000 $\mu\text{g}/\text{ml}$, and 1% and 10% w/v. Incubations using conjugate (goat anti-human IgG bound to alkaline phosphatase) and substrate (*p*-nitrophenyl phosphate) were conducted as before.

Inhibition of concanavalin A surface binding by purified antigen

Aliquots of approximately 10–15 *S. mansoni* adult worms were incubated at 37°C for 30 min in Earle’s balanced salt solution (without phenol red indicator) containing fluorescein isothiocyanate-conjugated (FITC) Con A (E.Y. Laboratories, San Mateo, California) both with and without the addition of the purified 58,000-molecular-weight glycoprotein. Incubation was carried out using 25 $\mu\text{g}/\text{ml}$ and 2.5 $\mu\text{g}/\text{ml}$ FITC-Con A. Antigen concentrations were 180 $\mu\text{g}/\text{ml}$ and 0 $\mu\text{g}/\text{ml}$ (control). After incubation, worms were washed three times in 10 ml Earle’s salts (without phenol red indicator) at room temperature. Worms were placed on a glass slide containing one drop of Earle’s salts, and were examined using a Nikon “Optiphot” fluorescent microscope at 530 nm. Slides were scored subjectively using a scale of – to +++++.

Reproducibility

Antigen fractionation by affinity chromatography, ampholine isoelectric focusing, and SDS-PAGE was repeated over a dozen times with similar, if not identical, results. Enzyme-linked immunoassay (EIA) was also found to be routinely repro-

ducible; assays described in this paper were performed at least three times. Data presented in the figures and tables are, in each case, the results of a single representative experimental run. Inhibition of FITC-Con A surface binding by antigen was done only once to conserve limited amounts of the purified antigen.

Buffers

ACETATE BUFFER: 0.1 M sodium acetate to which was added 0.5 mM Ca^{++} and Mn^{++} (99 mg/liter $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ and 56 mg/liter anhydrous CaCl_2); pH was adjusted to 6.8 by the addition of 1 N acetic acid.

BORATE BUFFER FOR RADIOLABELING: 0.1 M borate buffer prepared using 6.18 g/liter boric acid and 0.50 g/liter NaOH; pH was adjusted to 8.5 by the addition of approximately 5 ml 1 N NaOH.

BORATE BUFFER FOR ELUTION: 0.45 M borate buffer with molarity calculated as boron equivalents (following Svensson et al., 1970) prepared using 42.86 g/liter sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) and adjusted to pH 6.0 by the addition of approximately 56 g/liter solid KH_2PO_4 .

BORATE BUFFERED SALINE (BBS): 0.15 M BBS prepared using 11 g/liter boric acid and 6.3 g/liter NaCl; pH was adjusted to 7.6 by the addition of 1 N NaOH.

DETERGENT SOLUTION: 0.05% v/v Nonidet P-40 (non-ionic) detergent prepared in BBS containing 5 ml/liter aprotinin enzyme inhibitor (Trasylol®).

ELECTROPHORESIS BUFFER: 14.4 g/liter glycine, 3 g/liter Tris, and 1 g/liter sodium dodecyl sulfate (SDS), pH 8.0.

GLYCINE SOLUTION: 1% glycine prepared in 0.1 M borate buffer, pH 8.5.

HANKS' BALANCED SALT SOLUTION (Hanks' BSS): prepared commercially by GIBCO, Grand Island, New York.

SAMPLE BUFFER (for SDS-PAGE): 0.04 M Tris-HCl (pH 7.0), containing 2% w/v SDS, 20% v/v glycerol, 50 mM dithiothreitol (DTT), and 20 $\mu\text{g}/\text{ml}$ bromophenol blue (BB).

SUGAR SOLUTION FOR ELUTION: 0.1 M α -methylmannoside prepared in 0.1 M acetate (pH 6.8), containing 0.5 mM Ca^{++} and Mn^{++} (as above).

Results

Analytical separation using radiolabeled material

Bolton-Hunter radioiodinated freeze-thaw antigen in acetate buffer was applied to a 0.5×10 -cm Con A-Sepharose 4B column; the column was thoroughly washed, then eluted with 0.1 M α -methylmannoside to yield a single peak. Analysis of the labeled eluate by SDS-PAGE gave variable results: between one and four peaks might be observed with material eluted in a given experimental run. A peak of approximately 58,000 molecular weight was found in all instances; in some experiments, peaks of approximately 30,000, 94,000, and 150,000 molecular weight were also detected (Fig. 1A). In order to rule out nonspecific adsorption by Con A-Sepharose, the eluate was dialyzed (to remove the α -methylmannoside) and then run on a second Con A column. The α -methylmannoside eluate from the second column was comprised primarily of the 58,000-molecular-weight peak (Fig. 1C), although minor peaks persisted. This was true for both unreduced samples and samples reduced using 50 mM dithiothreitol (DTT). Fractionation of the eluate by ampholine isoelectric focusing revealed a single peak with a pI

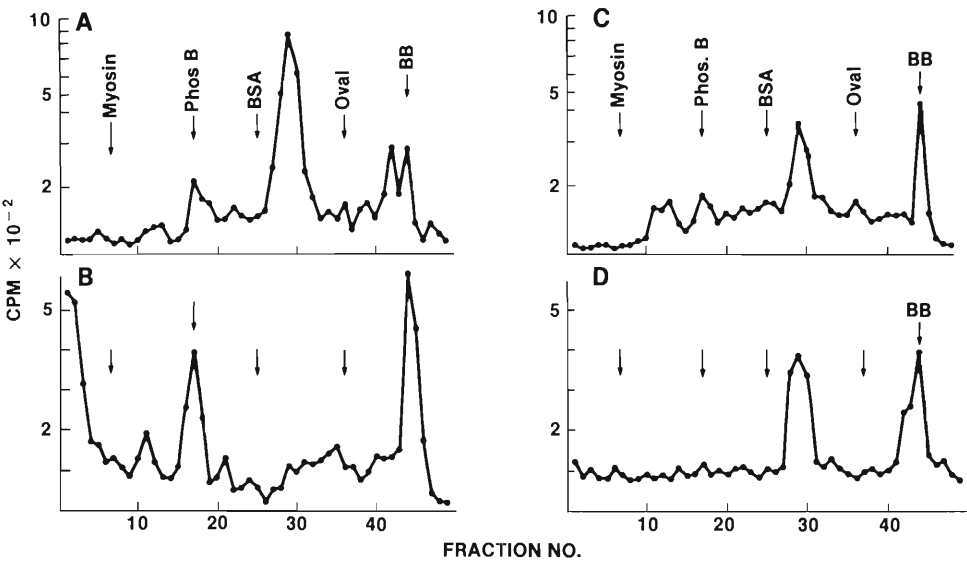


Figure 1. SDS-PAGE profile of radiolabeled *Schistosoma mansoni* proteins after separation by lectin affinity chromatography, 7½% gels, 2-mm slices. A. Alpha-methylmannoside eluate of Bolton-Hunter labeled freeze-thaw antigen separated by Con A-Sepharose 4B. B. Eluate of freeze-thaw antigen separated by lentil lectin-Sepharose 4B. C. Eluate of freeze-thaw antigen separated by two successive Con A columns. D. Eluate of detergent extracted tegumental antigen separated by Con A-Sepharose 4B.

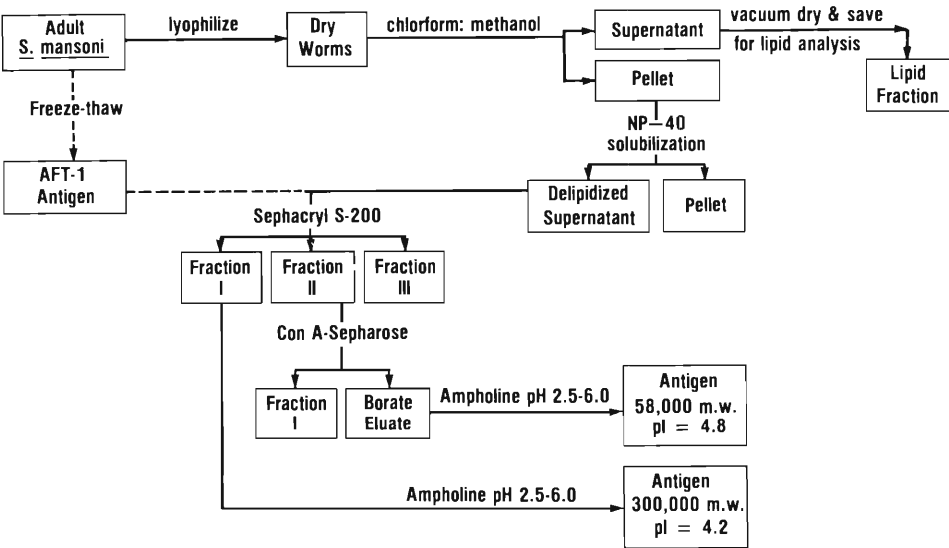


Figure 2. Flow diagram depicting the fractionation protocol for purifying the Con A-binding antigen from *Schistosoma mansoni* adult worms.

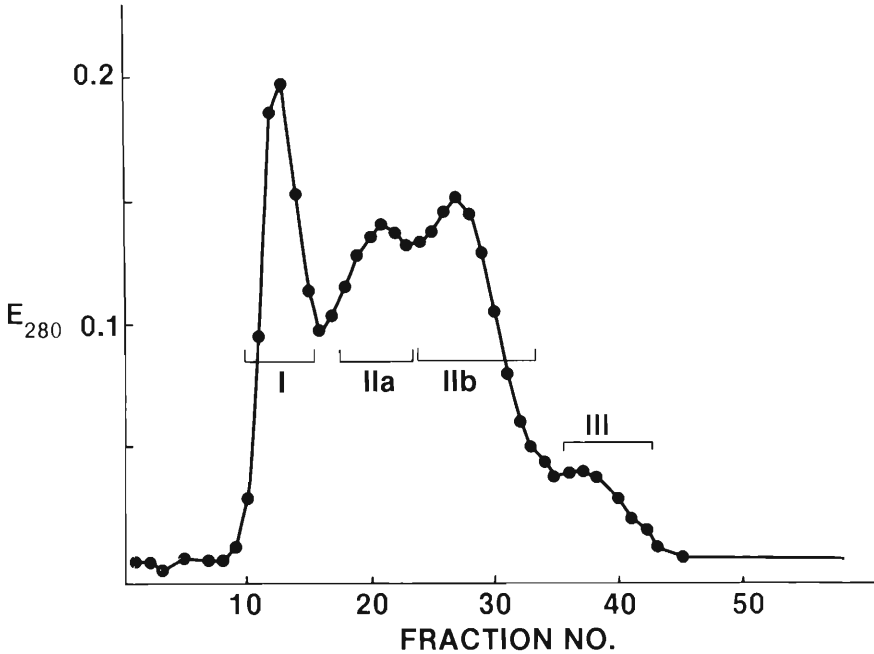


Figure 3. Fractionation of antigen from lyophilized adult worms by gel filtration using Sephacryl S-200 Superfine.

value of approximately 4.8. The ampholine peak, in turn, yielded a single peak when run on SDS gels. Thus, we concluded that the 58,000-molecular-weight component was the major Con A-binding glycoprotein extracted from *S. mansoni* adult worms. The minor peaks may have represented (a) glycoproteins that react only weakly with Con A, (b) labile Con A-binding glycoproteins that are unable to withstand excessive handling, or (c) material nonspecifically adsorbed by the column.

Tegumental material extracted by incubation of worms in 0.05% Nonidet P-40 detergent was also labeled and analyzed. SDS gels of the α -methylmannoside eluate of this material showed only a single peak of approximately 58,000 molecular weight (Fig. 1D). This would suggest that the 58,000-molecular-weight component is the major Con A-binding site associated with the parasite's surface.

For comparison, radiolabeled freeze-thaw antigen was applied to a lentil lectin-Sepharose 4B column, and the α -methylmannoside eluate was analyzed by SDS-PAGE. As shown in Figure 1B, this procedure yielded a single peak of approximately 94,000 molecular weight; the 58,000-molecular-weight peak that bound to concanavalin A did not react with lentil lectin. Data from affinity chromatography using other lectins will be described in detail elsewhere.

Isolation and purification of the antigen

Starting with lyophilized *S. mansoni* adult worms, the isolation protocol involves chloroform: methanol extraction, Sephacryl S-200 gel filtration, Con A-Sepharose 4B affinity chromatography, and ampholine isoelectric focusing (Fig.

Table 1. Amount of purified glycoprotein isolated from *Schistosoma mansoni* adult worms.

	Con A-binding glycoprotein	High-molecular-weight glycoprotein
4,000 adult <i>S. mansoni</i>	5 ml	5 ml
Lyophilized worms	275 mg	275 mg
Pellet after chloroform : methanol extraction	215 mg	215 mg
Detergent extract	5 mg	5 mg
Sephacryl "Fraction I"	—	1.9 mg
Ampholine peak, pI = 4.27	—	~350 µg
Sephacryl "Fraction II"	2.7 mg	—
Concanavalin A eluate	300 µg	—
Ampholine peak, pI = 4.85	~40 µg	—

Table 2. Enzyme-linked immunoassay (EIA) to detect reactivity of crude and purified antigen preparations. Data expressed as absorbance at 405 nm after 1-hr incubation with *p*-nitrophenyl phosphate substrate.

Sera (1:100 dilution)	Antigen preparations*						Serum blank (no antigen)
	<i>S. mansoni</i> AFT-1 (4 µg/ml)	<i>S. mansoni</i> AFT-1 (8 µg/ml)	<i>S. mansoni</i> AFT-1 (10 µg/ml)	<i>S. mansoni</i> Con A (2 µg/ml)	<i>S. mansoni</i> Con A (4 µg/ml)	<i>S. japonicum</i> Con A (4 µg/ml)	
<i>S. mansoni</i> infection serum	0.482	0.673	0.643	0.344	0.408	0.628	0.107
<i>S. haematobium</i> infection serum	0.304	0.502	0.571	0.360	0.431	0.576	0.086
<i>S. japonicum</i> infection serum	0.277	0.293	0.324	0.499	0.522	0.656	0.083
Normal human serum	0.067	0.063	0.080	0.090	0.098	0.113	0.037
Antigen blank (no serum)	0.070	0.056	0.079	0.055	0.053	0.085	0.038

* *Schistosoma mansoni* freeze-thaw antigen (AFT-1) prepared following Murrell et al. (1974); *S. mansoni* Con A-binding antigen described in the present paper; *S. japonicum* Con A-binding antigen prepared following Hayunga et al. (1982).

Table 3. Enzyme-linked immunoassay (EIA) to detect reactivity of crude and purified antigen preparations. Data expressed as absorbance at 405 nm after 90-min incubation with *p*-nitrophenyl phosphate substrate.

Sera (1:100 dilution)	Antigen preparations*					Serum blank (no antigen)
	<i>S. mansoni</i> AFT-1 (4 µg/ml)	<i>S. mansoni</i> AFT-1 (8 µg/ml)	<i>S. mansoni</i> Fx I (2 µg/ml)	<i>S. mansoni</i> Fx I (4 µg/ml)	<i>S. mansoni</i> Fx I (8 µg/ml)	
<i>S. mansoni</i> infection serum	0.563	0.615	0.483	0.598	0.785	0.107
<i>S. haematobium</i> infection serum	0.502	0.548	0.411	0.469	0.624	0.095
<i>S. japonicum</i> infection serum	0.314	0.348	0.376	0.433	0.556	0.106
Normal human serum	0.129	0.159	0.098	0.098	0.133	0.072
Antigen blank (no serum)	0.107	0.130	0.090	0.086	0.100	0.079

* *Schistosoma mansoni* freeze-thaw antigen (AFT-1) prepared following Murrell et al. (1974); *S. mansoni* large-molecular-weight antigen (Fx I) purified by ampholine column as described in the present paper.

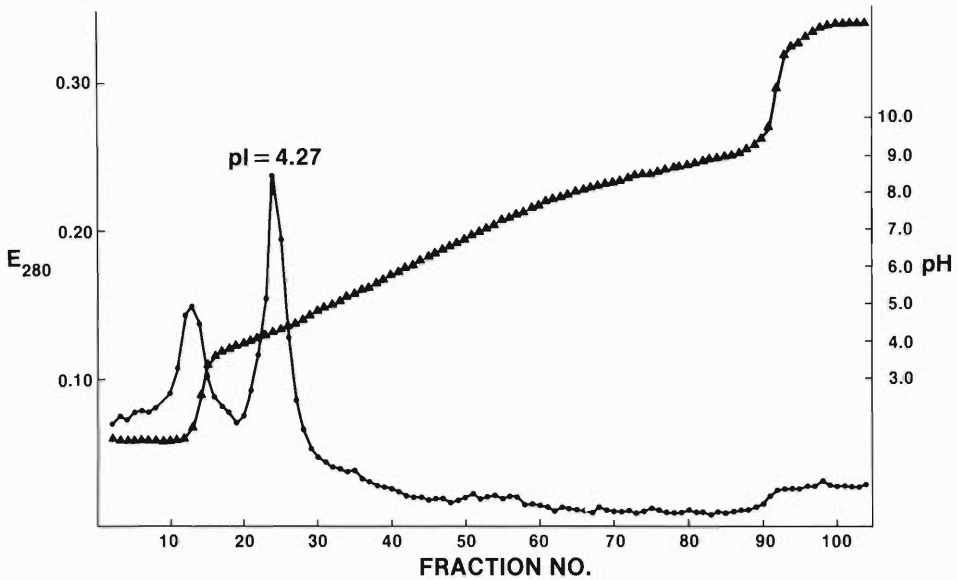


Figure 4. Fractionation of Fraction I from the Sephacryl gel filtration column by ampholine isoelectric focusing, pH gradient 3–10. Subsequent analysis of this peak by SDS-PAGE revealed a molecular weight of approximately $\geq 300,000$.

2). Although the antigen can also be isolated from freeze–thaw preparations (AFT-1), we found no increase in yield to justify the extra effort involved in making AFT-1.

Approximately 4,000 lyophilized adult worms (=approximately 275 mg, dry weight) were resuspended in 25 ml chloroform : methanol (2:1) and disrupted in a ground-glass tissue homogenizer. After 2 hr extraction at room temperature, the mixture was centrifuged at 3,000 *g* for 30 min and the supernatant removed; a second extraction was done overnight at 4°C. This procedure removed approximately 60 mg of material. The pellet was dried, then resuspended in 5 ml 0.5% Nonidet P-40 (non-ionic) detergent in 0.1 M acetate buffer, incubated for 30 min at 37°C, and centrifuged at 3,000 *g* for 30 min. The supernatant, which contained approximately 5 mg protein, was dialyzed against two changes of acetate buffer (to remove detergent), then applied to a 1.6×25 -cm Sephacryl S-200 column at a flow rate of 3 ml/hr.

Gel filtration on the Sephacryl S-200 column yielded the elution profile shown in Figure 3. Fraction I consisted of the large-molecular-weight (approximately 300,000) yellowish material that we believe had interfered with initial attempts at preparative affinity chromatography (see discussion). Analysis of this fraction by ampholine isoelectric focusing (Fig. 4) revealed a single peak with an approximate pI value of 4.2 (range 4.0–4.4). The component that we sought to purify (molecular weight = 58,000) eluted around the “valley” between Fractions IIa and IIb (Fig. 3). Fractions IIa and IIb (which together contained approximately 2.7 mg protein) were pooled and applied directly to a preparative (0.9×20 cm) Con A–Sephacryl 4B column, washed with acetate buffer, then eluted with 0.45 M borate to yield a single peak.

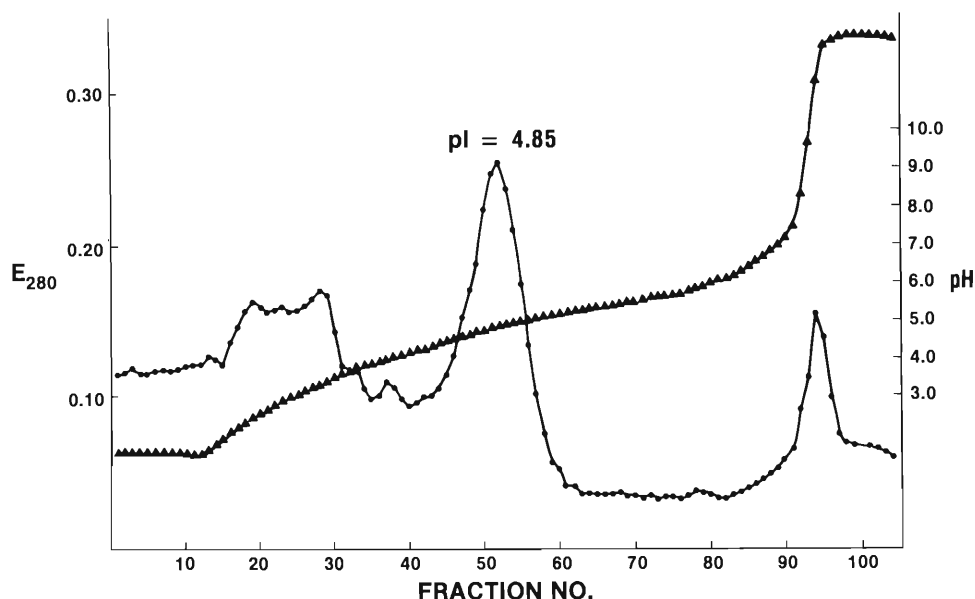


Figure 5. Fractionation of borate eluate from the Con A column by ampholine isoelectric focusing, pH gradient 2.5–6.0. Subsequent analysis of this peak by SDS-PAGE revealed a molecular weight of approximately 58,000.

The eluate from the Con A column (approximately 300 μ g) was next dialyzed against distilled water to remove salt, then run on a 110-ml ampholine isoelectric focusing column, pH range 2.5–6.0. As shown in Figure 5, the glycoprotein migrated as a prominent peak, with a pI value of approximately 4.8 (range 4.6–4.9). The minor peaks on this figure probably represent contamination or products of enzymatic degradation that would not have been removed without the ampholine step.

From an initial amount of over 4,000 worms (5 ml packed volume, approximately 275 mg, dry weight), we were able to recover about 40 μ g of the Con A-binding glycoprotein and 350 μ g of the high-molecular-weight antigen (Table 1). Purity of either final product can be judged by migration as a single peak on the ampholine column (Figs. 4, 5).

Reactivity with antisera

Purified antigens were compared to crude freeze-thaw (AFT-1) antigen for their ability to react with antisera in the enzyme-linked immunoassay (EIA). The Con A-binding antigen from *S. mansoni* was found to react with sera from patients infected with *S. mansoni*, *S. haematobium*, or *S. japonicum* (Table 2). In such a comparison, interpretation must be guarded because quantitative results may simply reflect differences in serum titers. However, it would appear that this antigen is more cross-reactive than the crude (AFT-1) preparation. Similarly, a Con A-binding antigen isolated from *S. japonicum* (Table 2) and the large-molecular-weight antigen isolated from *S. mansoni* (Table 3) were also highly cross-

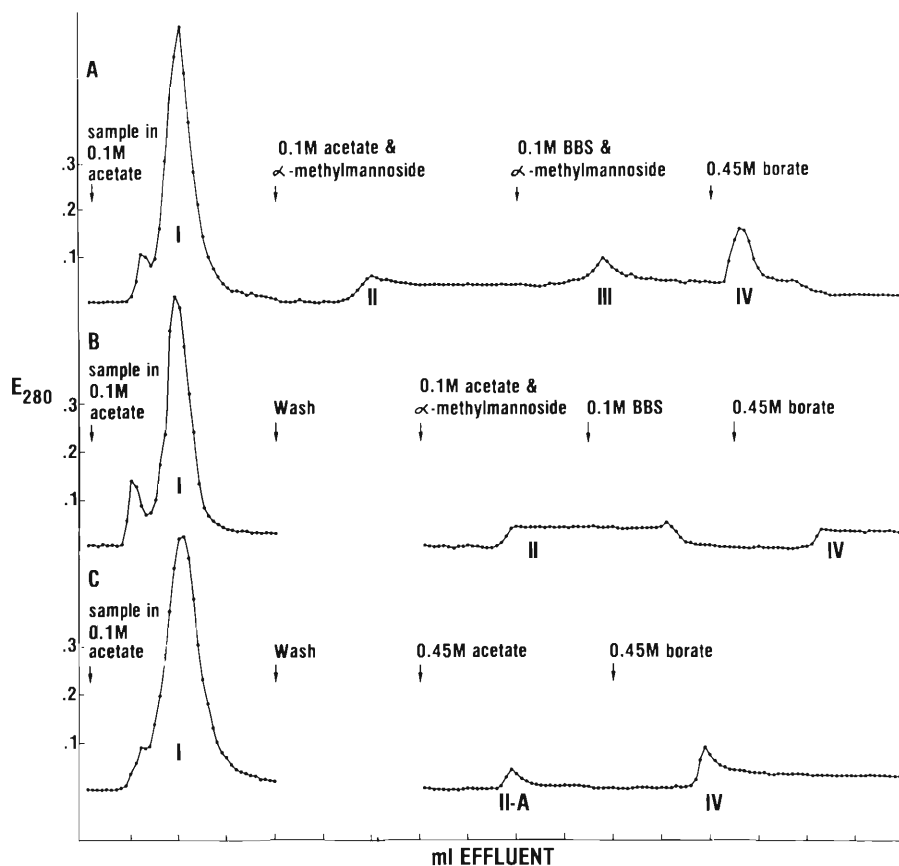


Figure 6. Elution profile of freeze-thaw antigen separated by preparative Con A-Sepharose 4B column chromatography. A. Initial separation protocol. B. Modification of protocol by adding a washing step prior to elution. C. Final protocol using borate instead of α -methylmannoside for elution.

reactive. Reaction with any of the infection sera was significantly greater than with normal serum.

Reaction of *S. mansoni* infection serum with the Con A-binding glycoprotein could be inhibited by the addition of any of several monosaccharides (Table 4). Inhibition was obtained using 10% fucose, mannose, or α -methylmannoside, but not with glucose. Reduction of antibody binding also occurred with galactosamine and glucosamine. Inhibition occurred only at very high concentrations, and in no instance was 100% inhibition of antibody binding achieved.

Inhibition of Con A surface binding

Fluorescein isothiocyanate-conjugated concanavalin A (FITC-Con A) was found to bind the surface of *S. mansoni* adult worms. The reaction was greatest after 30 min incubation with 25 μ g/ml FITC-Con A; a weaker positive reaction could also be obtained using a concentration of 2.5 μ g/ml. At either concentration, addition of the 58,000-molecular-weight glycoprotein to the incubation medium was found to reduce surface binding of the labeled lectin (Table 5). Although we

Table 4. Modified enzyme-linked immunoassay (EIA) to detect inhibition of antibody binding by various carbohydrates. Data expressed as absorbance at 405 nm after 30-min incubation with *p*-nitrophenyl phosphate substrate.*

Sugar	Concentration (in µg/ml)							Serum blank (no antigen)
	0	1	10	100	1,000	10,000 (1%)	100,000 (10%)	
glc	0.521	0.458	0.463	0.546	0.540	0.563	0.531	0.031
glcNH	0.593	0.525	0.449	0.496	0.572	0.499	0.268	0.036
glcNAc	0.520	0.445	0.423	0.537	0.480	0.432	0.349	0.035
gal	0.561	0.559	0.492	0.505	0.498	0.466	0.397	0.032
galNH	0.565	0.507	0.534	0.522	0.492	0.527	0.247	0.041
galNAc	0.538	0.523	0.519	0.472	0.497	0.475	0.335	0.037
fuc	0.456	0.398	0.371	0.423	0.346	0.281	0.211	0.015
man	0.552	0.521	0.401	0.506	0.389	0.427	0.224	0.028
Me-α-man	0.512	0.476	0.382	0.338	0.445	0.454	0.174	0.029
Antigen blank (no serum)	0.036	0.037	0.031	0.027	0.029	0.030	0.033	0.032

* Antigen: *S. mansoni* Con A-binding antigen, 4 µg/ml. Antibody: human *S. mansoni*-infection serum, diluted 1:100.

did not have sufficient purified antigen to perform a complete titration series, Con A binding was clearly inhibited at the antigen and lectin concentrations examined.

Discussion

Despite their potential for improved serodiagnosis and vaccine development and their importance in basic immunological research, there has been little progress in isolating and purifying surface antigens from *S. mansoni*. Nash et al. (1977) isolated a circulating proteoglycan secreted by the gut of adult worms, and soluble egg antigen (SEA) has been well defined (Boros and Warren, 1970; Pelley et al., 1976; Carter and Colley, 1978). However, attempts to harvest antigens from the tegument have yielded very heterogeneous mixtures containing protein, glycoprotein, and lipid (Murrell et al., 1974). The procedure described in this paper represents the first quantitative isolation of a single surface membrane or tegumental antigen from *S. mansoni* adult worms.

Technical considerations

As shown in the flow diagram in Figure 2, the Con A-binding glycoprotein may be isolated from fresh, frozen, or lyophilized worms. However, yields are comparatively poor, because the glycoprotein represents less than 1% (by weight) of

Table 5. Inhibition of concanavalin A surface binding* by incubation of *Schistosoma mansoni* adult worms with fluorescein-labeled lectin (FITC-Con A) and purified glycoprotein antigen.

		Concentration of antigen	
		0 µg/ml (control)	180 µg/ml
25 µg/ml	FITC-Con A	++++	++
2.5 µg/ml	FITC-Con A	+	—

* Surface binding scored as: positive reaction (+), stronger reaction (++), very strong reaction (+++), extremely strong reaction (++++), no reaction (—).

the water-soluble proteins extracted by either freeze-thaw or detergent. Initial attempts to isolate the 58,000-molecular-weight antigen by affinity chromatography were fraught with difficulties. When approximately 20 mg of unlabeled freeze-thaw (AFT-1) antigen was applied directly to a 0.9×20 -cm Con A column, the α -methylmannoside eluate appeared as a very low broad peak (Fig. 6A, Fraction II). As an alternative, we eluted the column using borate buffer, because borate inhibits Con A binding by forming complexes with the 4- and 6-hydroxyls of nonreducing pyranosides in glycoproteins (Svensson et al., 1970; Kennedy and Rosevear, 1973). Although increasing concentrations of borate buffer eluted more material from the column (Fig. 6A, Fractions III and IV), SDS gels of this material revealed numerous protein bands, suggesting nonspecific adsorption of freeze-thaw antigen by Sepharose and elution due to an increase in ionic strength of the buffer. Washing the column with 500 ml acetate buffer before elution reduced the yield (Fig. 6B), but the eluate was comprised primarily of the 58,000-molecular-weight antigen. A final modification of the protocol, without α -methylmannoside elution, is shown in Figure 6C. After washing with 0.1 M acetate buffer, an increase in ionic strength (to 0.45 M acetate) removes additional impurities. Fraction IV represents material eluted by interaction with the borate ion.

However, results were complicated by nonspecific and irreversible binding of crude antigen to Con A-Sepharose. When one of the preparative (0.9×20 cm) Con A columns was used a second time, the yield was markedly diminished. After three or more sample applications, the column took on a permanent yellow-brown color; successive washings with sodium acetate (pH 4.5) to "regenerate" the column failed to elute the contaminant. Both chloroform : methanol extraction and gel filtration remove yellow-colored material so that a more defined sample may be applied to the Con A column.

Biochemical characterization of the Con A-binding antigen

Throughout the text, we have described the Con A-binding antigen as having a molecular weight of 58,000. This value can only be taken as a rough approximation based upon relative mobility in SDS-PAGE. It is well documented that the carbohydrate moieties of glycoproteins can greatly alter the intrinsic net charge of the molecule (Poretz and Pieczenik, 1981) and cause migration abnormalities of up to 33% in SDS gels (Glossmann and Neville, 1971; Weber and Osborn, 1975). Because we have not yet determined the total amount or identity of carbohydrates in the glycoprotein, it would be premature to speculate about its actual molecular weight.

The ability of the glycoprotein to bind concanavalin A but not *Lens culinaris* (lentil) lectin is problematic, because it is generally assumed that both lectins react with α -D-glucosyl or sterically related carbohydrate groups. Although a number of glycopeptides will react with both Con A and *L. culinaris*, there are significant differences in association constants between the two (Young and Leon, 1974). It appears that the actual binding site of Con A accommodates structures as large as di- or trisaccharides; concanavalin A has greatest affinity for "mannobiosyl-N-acetylglucosamine," while "N-acetylglucosaminylmannobiose" is the structure recognized by *L. culinaris* (Toyoshima et al., 1972; Young and Leon, 1974). Because these structures are similar, we would expect at least some binding of the glycoprotein with *L. culinaris* as well as with Con A. Failure of the antigen

to react with both may be due to our use of immobilized lectins. As observed by Kennedy and Rosevear (1973), "molecules of soluble Con A are free to move in relation to each other Immobilization of the protein would prevent this movement and consequently only carbohydrates with a particular distance between nonreducing ends would interact with the maximum possible strength In the immobilized case, molecules may be fractionated according to differences in the length of branches or the distribution of branch points which are not apparent when the complexation is carried out in solution." Thus, separation of the 58,000-molecular-weight Con A-binding glycoprotein from the 94,000-molecular-weight lentil lectin-binding glycoprotein (compare Fig. 1A and B) may reflect differences in the steric arrangement of the lectin molecules bound to Sepharose beads rather than major differences in exposed carbohydrate residues of the glycoproteins.

We believe that mannose, or a structurally similar saccharide, is present as a terminal or penultimate carbohydrate in the Con A-binding antigen. Reactivity with Con A but not with wheat germ agglutinin suggests terminal glucosamine but not N-acetylglucosamine. Metabolic labeling using tritiated precursors revealed significant incorporation of galactose in the 58,000-molecular-weight antigen; detection of other labeled sugars has not yet been conclusive. Elucidation of the complete carbohydrate composition and sequence of branch chains in the glycoprotein must await further analysis.

Immunochemical characterization of the Con A-binding antigen

Localization of the glycoprotein on the surface of the parasite is inferred by previous studies using fluorescein-labeled lectins, which showed that Con A binding was restricted to the surface (Simpson and Smithers, 1980). Inhibition of Con A binding by the glycoprotein (Table 5) argues strongly that it is a surface antigen. Further support is gained from isolation of the glycoprotein by detergent extraction, because low concentrations of non-ionic detergent appear to remove only tegumental material (Brink et al., 1980; Oaks et al., 1981; Hayunga and Murrell, 1982). Conclusive proof of surface localization would require electron-microscopic histochemistry.

Cesari (1974) and Bennett and Seed (1977) speculated that Con A-binding material from adult worms might represent a tegumental alkaline phosphatase. The glycoprotein isolated in this study failed to give a colorimetric reaction when incubated with *p*-nitrophenyl phosphate substrate at alkaline pH; either it was not a phosphatase or enzymatic activity was lost as a result of the isolation procedure.

When used as a coating antigen for enzyme-linked immunoassay (EIA), both the Con A-binding glycoprotein and the large-molecular-weight antigen from *S. mansoni* reacted with sera from patients infected by *S. haematobium* or *S. japonicum* as well as those infected by *S. mansoni*. Similarly, the glycoprotein isolated from *S. japonicum* adult worms (Hayunga et al., 1982) also reacted with all three antisera. Thus, in terms of practical application, purified antigens were for the most part as sensitive as crude antigen (AFT-1) in EIA, but none of them could be considered a species-specific antigen for serodiagnosis. In a previous study, the Con A-binding glycoprotein from *S. mansoni* schistosomula was found

to have a molecular weight significantly different from 58,000 (Taylor et al., 1981), which suggests that the glycoprotein isolated from adult worms may represent a stage-specific antigen.

Inhibition of glycoprotein-antibody binding by monosaccharides was significant only at concentrations of 1% and 10% (Table 4). Although high, these concentrations are comparable to those used to inhibit lectin binding to the tegument of *S. mansoni* (Bennett and Seed, 1977) and to inhibit both lectin binding (Goldstein et al., 1965; Young and Leon, 1974) and antibody binding (Cisar et al., 1974, 1975) to dextran. The results described in this paper suggest that antibody-binding sites on the 58,000-molecular-weight glycoprotein most likely contain moieties that are structurally similar to mannose, fucose, glucosamine, and galactosamine. In contrast, the lack of inhibition by glucose indicates that there are strict steric requirements for antibody binding. Fucose (6-deoxy-galactose) was a more efficient inhibitor than galactose. The 2-amino sugars were more efficient than were their N-acetyl derivatives, suggesting steric hindrance of an amino group important in recognition. Both the amino and N-acetyl sugars were more efficient inhibitors of antibody binding than were their parent sugars. Doubtless, the actual antibody-binding sites on the glycoprotein are complex, involving a number of saccharides in a precise sequence and linkage. There may be several different antibody-binding sites on the antigen, and binding sites for antibodies may not necessarily be identical to those for lectins. Monoclonal antibodies derived from hybridomas would be invaluable tools to elucidate the number and types of binding sites on this antigen.

Recently, Hillyer and Sagramoso de Ateca (1979) have isolated a Con A-binding glycoprotein from the tegument of *Fasciola hepatica* that not only reacts with *S. mansoni* infection serum but that gives partial protection against murine schistosomiasis. With an apparent molecular weight of 60,000 and pI value 4.0–4.4, this antigen bears a striking resemblance to the Con A-binding glycoprotein that we have isolated from *S. mansoni* adult worms. Preliminary experiments in our laboratory suggest that the *S. mansoni* glycoprotein may also have some effect in protecting mice against infection. Clearly, further characterization is needed for what appear to be common surface antigens in two distantly related trematodes.

Isolation of other antigens

As a by-product of our efforts to purify the Con A-binding glycoprotein from *S. mansoni*, we also isolated a large (approximately 300,000)-molecular-weight antigen and an uncharacterized lipid fraction. The large-molecular-weight antigen was found to have a pI value of approximately 4.2 (range 4.0–4.4) as determined by ampholine isoelectric focusing (Fig. 4), and it reacted strongly with infection serum (Table 3). The nominal molecular weight of 300,000 is a rough approximation based on gel filtration; the antigen barely penetrated the stacking gel in SDS-PAGE. A slight, but statistically significant, reduction of antibody binding (data not shown) was found with each of the nine monosaccharides used for the inhibition test, which is suggestive of a large glycoprotein with complex arrangement of saccharide chains. Thus, the antigen may correspond to one of the glycoproteins of similar molecular weight found in preparations of culture antigens (Murrell et al., 1974), membrane antigens (Murrell et al., 1977), or detergent

extracts of the tegument (Hayunga et al., 1979). Further analysis of this antigen and the lipid fraction is currently under way.

Acknowledgments

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Erratum

The surname of the first author was misspelled for the paper “*Vampirolepis schmidtii* sp. n. (Cestoidea: Hymenolepididae) from *Triaenops persicus* (Hipposideridae) of Tanzania,” *Proc. Helminthol. Soc. Wash.* 50:135–137. The name should read “Jensen.” It is hoped that this correction will eliminate the possible confusion in the citing of this paper.

Aphodius* Beetles as Biological Control Agents of Elk Lungworm, *Dictyocaulus hadweni

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ABSTRACT: Laboratory experiments were designed to study the interaction of first-stage larvae of *Dictyocaulus hadweni* and adult *Aphodius* spp. beetles on or in elk fecal material. Feces were placed on moistened filter paper that had been placed on sterile soil 3 cm deep in glass finger bowls 20 cm in diameter by 8 cm deep. The tops of the bowls were covered by plastic screen to prevent escape of beetles. At laboratory temperatures of 4°C at night to 20°C during the day, one or two beetles per gram of feces caused reduction of *Dictyocaulus* larval numbers by 77–92% within 4–36 hr.

The lungworm, *Dictyocaulus hadweni* Chapin, 1925 (syn. in part *D. viviparus* (Bloch, 1782)) is an important parasite of elk in the Teton area of Wyoming (Alderson, 1949; Honess and Winter, 1956; Bergstrom, 1975) and adjacent areas (Stelfox, 1962, Alberta, Canada; Barrett and Worley, 1966, Montana) but less so farther south (Wilson, 1969, New Mexico). Although the lungworm infections are usually not lethal, most infected elk lungs show pathologic changes. Atelectasis and emphysematous areas are noted in practically all adult elk lungs from the Teton Park herd and in those from elk of the National Elk Refuge, Jackson, Wyoming. Both sexes and all ages of elk are infected in late April and early May each year (except calves, which are usually not infected until they are 2½–4½ mo old). More elk calves would probably be infected and at an earlier age were it not for the action of scarabeoid beetles ingesting or mechanically removing first stage *Dictyocaulus* larvae from elk fecal material.

Small dung beetles were shown to be important biological control agents against trichostrongylid eggs in sheep and cattle feces (Bergstrom et al., 1976). Fincher (1973) indicated biological control of gastrointestinal parasites of livestock because the beetles buried the feces with parasite eggs and larvae. Fincher et al. (1971) showed that coprophagous beetles are attracted to feces of various animals. Waterhouse (1974) considered beetles' burying activities a form of biological control of dung, which, if not destroyed, could pose problems for livestock and humans through destruction of vegetation. Possible action of the beetles toward *Dictyocaulus* larvae has not been noted in published literature.

This report is an attempt to show the effect of *Aphodius* and *Canthon* beetles interacting with lungworm larvae in or on elk feces under laboratory conditions and (with limited observations) in the field.

Materials and Methods

Most fecal collections were made at or just after daybreak. The elk were located as they left grassy areas with sagebrush on glacial outwash substrate. The general area sampled was just west of the Snake River and 2–12 miles south of Signal Mountain. As the elk entered the cover of lodgepole pine and Douglas fir near the sagebrush areas, they stopped or slowed their pace, urinated and/or defecated, and subsequently moved deeper into the timber for bedding and rumination.

Twenty to 100 g of feces were collected from each pellet group in small plastic sacks. Later the fecal samples were transferred from the sacks to plastic Petri dishes (9 cm diam), and the fecal pellets were rinsed with a jet of water from a dispensing bottle. After about 10 min the pellets were thoroughly rinsed again and removed by small tweezers from the water remaining in the Petri dish. The dish, which had previously been divided into six equal sectors by use of a felt tip marking pen, was placed under a stereo dissecting scope for inspection of the liquid and fecal debris, where the motile larvae were found. All larvae were counted and collected from the liquid by filtration. A few thousand larvae were kept on filter paper for further research. Fecal samples positive for relatively large numbers of *Dictyocaulus* larvae were stored in plastic sacks at 1–4°C until exposed to adult and immature beetles. Beetle species used in the trials were *Aphodius vittatus*, *A. coloradensis*, *A. fimetarius*, *A. homisus* and *Canthon* sp. *Aphodius vittatus* and *A. coloradensis* were used in most of these trials, except in 1979, when all the species listed above were used.

Four- to 10-g fecal samples were used in the trials. The feces were placed on moistened filter paper that had been placed on sterile soil 3 cm deep in finger bowls 20 cm in diameter by 8 cm deep. The bowls were covered by plastic screen so that oxygen was available to the beetles and *Dictyocaulus* larvae, but neither could escape from the bowl. Laboratory temperatures varied from about 4°C at night to about 20°C at peak high temperatures during the afternoon. Some trials were made at temperatures of 24°C within unlighted incubating ovens at night to learn whether beetles were active at suitable temperatures in darkness.

After several laboratory trials, beetles frequently became lethargic and were replaced by “new” beetles from field collections of that day. Between the hours of 10:00 A.M. and 4:00 P.M. on most days beetles were active in the field and were easy to collect from elk feces or the soil beneath fecal material.

From four to nine trials were completed each year, 1976–1980. Numbers of beetles per gram of feces were maintained at relatively low levels (one or two beetles), which seemed to simulate the numbers of beetles noted on fresh elk feces in the field.

Results

As indicated in Table 1, the mean number of beetles per gram of feces was low. An average of 1.5 beetles per gram of feces, active for 4–36 hr, decreased the mean number of *Dictyocaulus* larvae in the feces by 84.7% (range 82–92%). Time of interaction of larvae and beetles was usually less than 24 hr, except in 1976. Except for the years of 1979 and 1980, the weight of elk feces sampled, with its complement of larvae, was 4.5 g (Table 1). Mean numbers of *Dictyocaulus* larvae on or in the feces varied considerably by month (many more larvae present in the spring fecal collections) and by year. The number of larvae recovered from the dishes after 4–36 hr, with and without beetle interaction, was never as large as the preliminary estimates of larvae per gram of fresh feces. Decreases in larval numbers and mean percent decreases each year were significant at the 0.05 level, and when data were pooled and analyzed as paired samples the decrease was significant at the 0.01 level (Student's *t* test, where a *t* value of 2.75 was needed for significance at the 0.01 level with 31 degrees of freedom and the value obtained here was 3.25).

Table 1. Decimation of first-stage *Dictyocaulus* sp. larvae on or in elk feces by *Aphodius* spp. beetles.

Year	No. trials	No. beetles/ g feces	Hr interaction of beetles and larvae	Size fecal sample (g)	Mean no. <i>Dictyo.</i> L_1 /g	No. L_1 recovered		% decrease in <i>Dictyo.</i> by beetle action
						Control	Principals	
1976	4	1.5	12–36	4.5	213	119	13.5	88.6
1977	6	2.0	12–22	4.5	59	31	7.2	77.0
1978	4	1.5	4–18	4.5	4.5	14	2.2	84.0
1979	9	1.0	4–18	7.4	22.4	143	26.4	82.0
1980*	9	1.5	6–24	3.2	15.4	33	2.7	92.0
						Mean decrease = 84.7		

* Four trials conducted in darkness.

Discussion

During May and August 1976, there were many *Dictyocaulus* larvae in or on the elk feces and numbers of larvae in the laboratory trials that year reflected those numbers. *Dictyocaulus* larvae were not completely eliminated by one or two beetles per gram of feces after 12–36 hr of interaction. During 1978, larval numbers on feces were relatively low and the beetles either eliminated all larvae or reduced their populations to nearly zero (Table 1). During 1977, 1979, and 1980, *Dictyocaulus* larval numbers were moderate and the beetles decreased larval numbers markedly in the limited numbers of trials completed during those spring and summer months.

None of the data from the laboratory trials illustrate the true value of the *Aphodius* beetles as biological control agents. With summer air temperatures at 21°C or above when an elk defecates, the beetles alight upon the pellets or appear from soil and litter in the immediate area within 15 sec. I tried to race the beetles to the freshly dropped feces on several occasions but invariably lost the race. For that reason and for easy access to foraging elk, all fecal sampling was completed near daylight, to collect a large number of fecal samples at timber–grassland interface areas and to collect at temperatures of 3–13°C, when beetle activity was of low order.

Beetles do not effect their decimatory action against *Dictyocaulus* larvae during the cool and cold months. Beetles became active at about 5°C during late May and continued foraging until early October. There is little or no beetle activity in April, when many adult elk are acquiring infective larvae of *Dictyocaulus* from vegetation. The major contribution of the beetles appears to be their activities when the elk are on summer ranges. Calves, therefore, may benefit markedly from beetle activities until fall elk migrations and rutting activities concentrate elk and pose infection pressure from *Dictyocaulus* larvae. Beetle activities wane by September–October.

In summary, *Aphodius* beetles are important biological control agents, but their activity is limited to late spring, summer, and early fall months, so that they alone never control dictyocaulosis of elk.

Acknowledgments

I am indebted to Dr. L. R. Maki for statistical analyses, to Barbara Werner and Pat Bergstrom for technical assistance in laboratory work, and to Dr. Kenneth

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Chloride Ions in Gastric Contents from Helminth-free Calves Repel Exsheathed Larvae of *Ostertagia ostertagi*

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ABSTRACT: Experiments compared how exsheathed infective larvae of *Ostertagia ostertagi* responded to equal concentrations of chloride ions from either sodium chloride or calf gastric contents. The first experiment tested how exsheathed larvae responded to sodium chloride and calf gastric contents both at 14 mEq Cl⁻/liter. The second experiment determined how the exsheathed larvae responded when both sodium chloride and calf gastric contents were diluted. The third experiment tested how the larvae responded when both test substances were heated. The fourth experiment compared how the larvae responded when both substances were stored over dry ice for up to 1 yr. The fifth experiment measured the response of the larvae to each repellent diffusing from a test agar block. The data indicated (1) both sodium chloride and gastric contents repelled exsheathed larvae of *O. ostertagi*; (2) both sodium chloride and gastric contents repelled the larvae at concentrations greater than 0.5 mEq Cl⁻/liter; (3) both sodium chloride and gastric contents repelled the larvae even if the test substances were heated; (4) both sodium chloride and gastric contents repelled the larvae even if the test substances were stored on dry ice at -60°C for 1 yr; (5) sodium chloride diffused through the agar at less than one-half the rate of gastric contents. The data suggested that chloride ions in calf gastric contents repelled exsheathed larvae of *O. ostertagi*.

Stringfellow (1981) reported that the gastric contents from the abomasum from helminth-free calves repelled exsheathed larvae of *Ostertagia ostertagi* on agar Petri plates. The data indicated that the repellent in the gastric contents was chloride ion (Cl⁻). The present experiments compared how exsheathed larvae of *O. ostertagi* responded to equal concentrations of chloride ions from either sodium chloride or calf gastric contents under different experimental conditions. The results reported herein provided further evidence that chloride ion was the repellent in the calf gastric contents.

Materials and Methods

The gastric contents used in these experiments were obtained from the abomasa of helminth-free 3-mo-old calves and were stored on dry ice in vials at -60°C. The calves were six control animals in which the chloride ion concentrations and pH were determined electrometrically relative to six calves each infected with 400,000 infective larvae of *O. ostertagi* (Table 1). All calves were killed and necropsied 24 days after infection (DAI). Biological materials were prepared and laboratory determinations were made as reported in Stringfellow (1981).

The bioassay and statistical methods described previously by Stringfellow (1978) are briefly presented here. The infective larvae were exsheathed with 1.25% Clo-rox-saline. A ring of 30 exsheathed larvae was placed on agar in Petri plates 2 cm from either a control 2% agar block or a 2% agar block containing the test substance. They migrated freely over the agar surface for 6 hr. Three Petri plates were run for each control or test substance. The data were tested with chi-square at the 0.05 level of significance by comparing the experimental and theoretical values. Only Petri plates with the same background pH were compared. The term "response," as used herein, indicates that the larvae either did or did not touch

Table 1. Chloride ion concentration and pH of gastric contents from six helminth-free 3-mo-old calves and six calves infected (24 DAI) with 400,000 larvae of *Ostertagia ostertagi*.

	Cl (mEq/liter)		pH	
	Uninfected	Infected	Uninfected	Infected
Mean \pm SD	135.2 \pm 59	130.8 \pm 6	2.8 \pm 1.1	7.0 \pm 0.15
Range	93–259	123–140.4	2.2–5.4	6.8–7.2

a control block or an agar block containing the test substance. Larvae were removed from the agar Petri plate if they touched the block in any way or for any length of time. The response of exsheathed infective larvae of *O. ostertagi* to control or test agar blocks was tested with the following experiments.

The first experiment tested the response of exsheathed larvae to either sodium chloride or calf gastric contents, both with concentrations of 14 mEq Cl⁻/liter. Some of the 2% agar was adjusted with concentrated hydrochloric acid to pH 2.3. Other agar simply solidified without adjustment, at pH 7.0. These agars were used to prepare both control and test agar blocks as well as Petri plates with a suitable chloride ion concentration and background pH. Test agar blocks with gastric contents were prepared as follows: 3 ml of filtered, thawed gastric contents from a helminth-free calf was diluted with 17 ml of 2% purified agar (pH 7.0) at 45–50°C. Both sodium and chloride ion concentrations were diluted about 6.6 times with the agar (6.6×14 mEq Cl⁻/liter = 92 mEq Cl⁻/liter actually measured in the calf). This mixture was stirred, cooled, distributed in Petri plates, and refrigerated at 4–8°C until used. Test agar blocks 0.5 cm square were cut from this agar. They were bioassayed on agar Petri plates at pH 7.0. The response of the larvae to them was compared with control agar blocks without gastric contents (pH 7.0) on agar Petri plates at pH 7.0. Sodium chloride was then incorporated into 2% agar at a concentration of 14 mEq Cl⁻/liter. The chloride ion concentrations were equal on the test agar block (pH 7.0) and the agar Petri plate (pH 2.3). Only the sodium ion stood out relative to the background agar. The response of the larvae to it was compared with a control agar block (pH 7.0) without sodium chloride on an agar Petri plate at pH 2.3. Sodium chloride was then incorporated into 2% purified agar at a concentration of 14 mEq Cl⁻/liter (pH 7.0). The test agar block was bioassayed on an agar Petri plate in which the pH was adjusted to 7.0. The response of the exsheathed larvae to it was compared to the response with the control agar block without sodium chloride (pH 7.0) on an agar Petri plate at pH 7.0. I considered this experiment to be a reasonable test for the chloride ion, and it was the basis for subsequent experiments.

The second experiment tested the concentrations at which both sodium chloride and calf gastric contents did not repel the exsheathed larvae of *O. ostertagi*. The test substances were diluted so that the chloride ion concentrations of both sodium chloride and gastric contents ranged from 14 to 0.003 mEq Cl⁻/liter. The concentrations of chloride ions of each set of test agar blocks (pH 7.0) were equal. The bioassay was carried out on 2% agar Petri plates at pH 7.0.

The third experiment compared how the larvae responded to the two repellents when they were heated. The test substances and the controls were heated for 3 min at specified temperatures ranging between 25 and 70°C. They were then

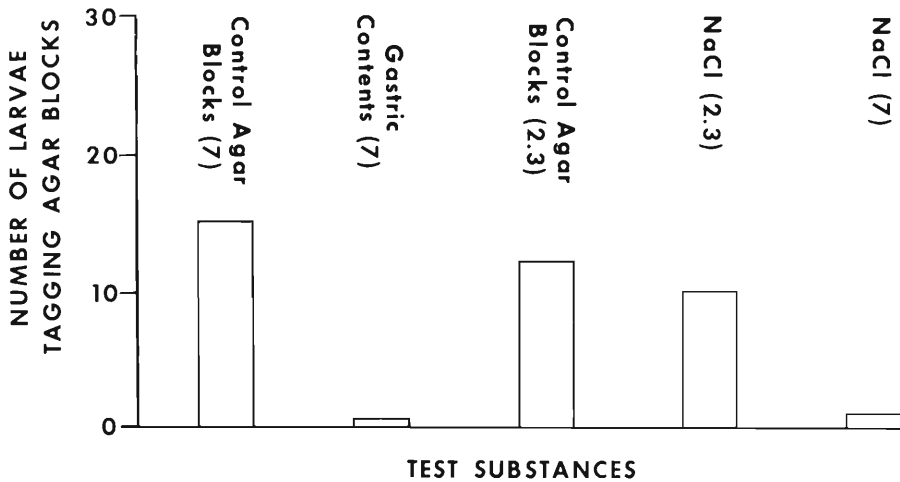


Figure 1. Histograms: response of exsheathed larvae of *Ostertagia ostertagi* to both control and test agar blocks. All control and test agar blocks were at pH 7.0. The numbers in parentheses indicate the pH of the agar Petri plates.

incorporated into 2% agar blocks. A test block consisted of either sodium chloride or gastric contents, each at a concentration of 14 mEq Cl⁻/liter; control agar blocks had no test substance. All agar blocks were at pH 7.0; all blocks were bioassayed on 2% agar Petri plates at pH 7.0.

Sodium chloride and gastric contents from uninfected calves were stored on dry ice for up to 1 yr at -60°C in the fourth experiment. They were thawed at intervals and each was incorporated into separate test agar blocks at 14 mEq Cl⁻/liter (pH 7.0). The response of the larvae to each of them was compared with a control agar block without the test substance (pH 7.0) on 2% agar Petri plates at pH 7.0.

The fifth experiment measured the response of the larvae to the repellent diffusing out of each test agar block. One thousand exsheathed larvae were pipetted onto an agar Petri plate (pH 7.0) at four opposing points about 2 cm from the center of the plate. Each 2% agar Petri plate was placed on a slide warmer at 37°C until the liquid surrounding the larvae evaporated. A control or a test agar block (pH 7.0) was placed at the center of each plate. Each test agar block consisted of either sodium chloride or gastric contents, both at concentrations of 14 mEq Cl⁻/liter; control agar blocks were without any test substance. The exsheathed larvae migrated freely for 3 hr at 18–20°C, and were observed with the aid of a dissecting microscope. The distance between the closest approach of the larvae to the agar blocks was measured with the aid of an ocular micrometer; data were recorded at 30-min intervals. Six intervals were replicated three times.

Results

The chloride ion concentration and the pH from both uninfected calves and those calves infected with terminal infections of *O. ostertagi* are summarized in Table 1. The gastric contents from abomasa from uninfected calves had a mean

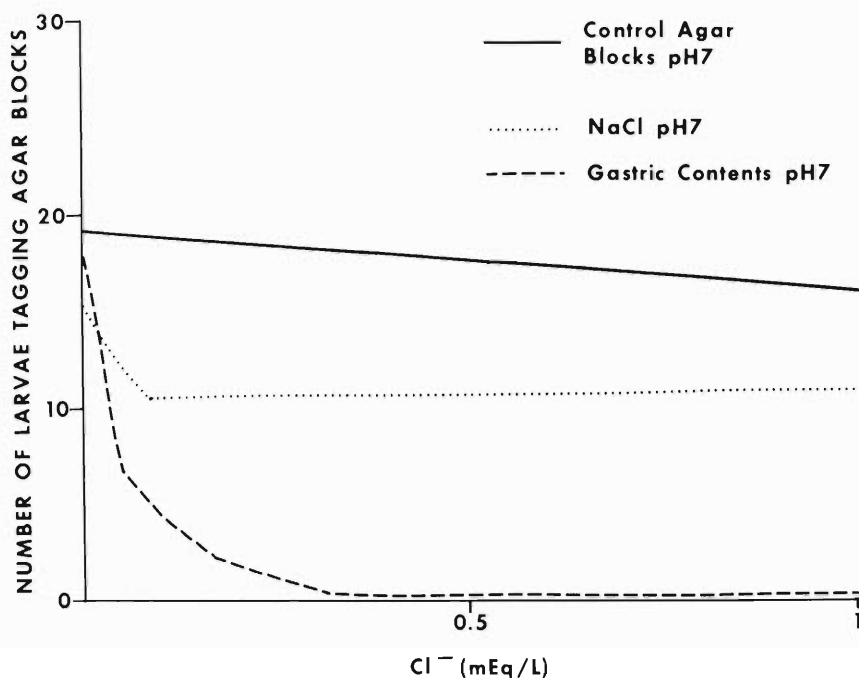


Figure 2. Line chart: number of exsheathed larvae of *O. ostertagi* responding to control and test agar blocks. The pH of all control and test agar blocks as well as the pH of the agar Petri plates was 7.0. The control agar blocks did not have the test substances with chloride ions. Each set of test agar blocks had decreasing and equal concentrations of chloride ions.

chloride ion concentration of 135 mEq Cl⁻/liter and a mean pH of 2.8. The difference in the average chloride ion concentration in the gastric contents between uninfected and infected abomasa was not significant, even in severe terminal infections where the pH of the gastric contents approached 7.0.

The results of the first experiment are shown in Figure 1. Significantly fewer larvae touched the test agar block (pH 7.0) with gastric contents than its agar block control (pH 7.0). Similarly, significantly fewer larvae responded to sodium chloride at pH 7.0 than to the control agar block at pH 7.0. These data suggested that the chloride ions in the gastric contents repelled the exsheathed larvae of *O. ostertagi*. In contrast, the larvae responded about the same to agar blocks containing sodium chloride as they did to their control agar blocks without it. The sodium ion stood out relative to the background agar because the concentration of the chloride ions was equal between both the test agar block and the agar of the Petri plate (pH 2.3). These data indicated that the larvae did not respond to the sodium ion. The results of the second experiment are shown in Figure 2. The exsheathed larvae were repelled by both sodium chloride and gastric contents at concentrations greater than 0.5 mEq Cl⁻/liter. The third experiment showed that both sodium chloride and gastric contents repelled the exsheathed larvae, compared with the control agar block, when they were heated (Fig. 3). The fourth experiment gave results similar to the data shown in Figure 1. Both sodium

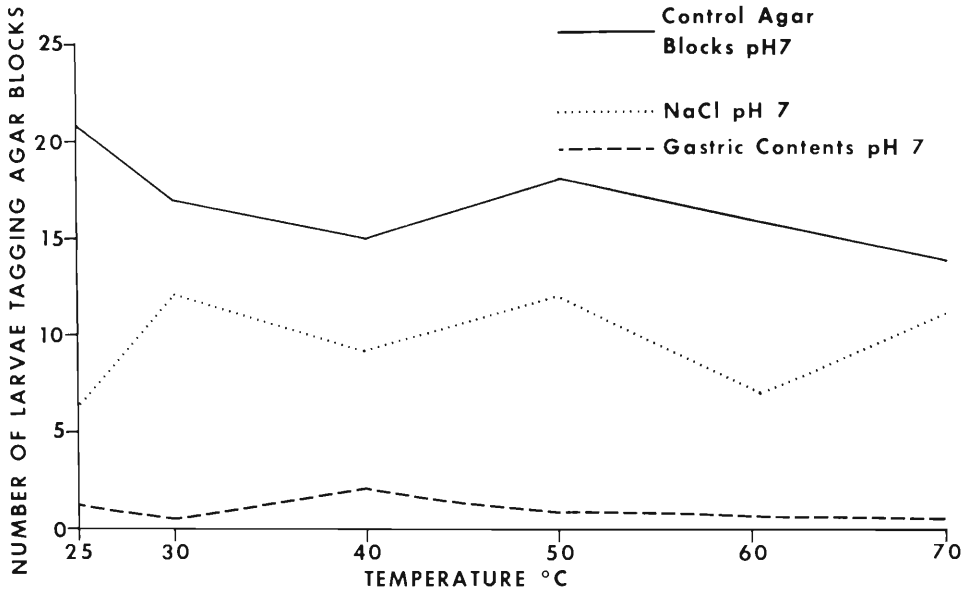


Figure 3. Line chart: number of exsheathed larvae of *O. ostertagi* responding to control and test agar blocks heated to 25–70°C. The pH of all control and test agar blocks as well as the pH of the agar petri plates was 7.0. The control agar blocks did not have the test substances with chloride ions. The test agar blocks had equal concentrations of chloride ions.

chloride and gastric contents repelled the exsheathed larvae even if they were stored on dry ice at -60°C for up to 1 yr. The fifth experiment measured the response of the larvae to each repellent diffusing from its test agar block. The results indicated that sodium chloride diffused through the agar at a little less than one-half the rate of gastric contents. Average rates of diffusion were (1) gastric contents: 2.1 mm/80 min; (2) sodium chloride: 1.0 mm/80 min; (3) control agar block: none. These different rates of diffusion might explain how the curves for both sodium chloride and gastric contents differed when superimposed on one another (Figs. 2, 3).

Discussion

It was concluded that calf gastric contents repelled exsheathed larvae of *O. ostertagi*. The data suggested that the chloride ion was the repellent. Chloride ions secreted by parietal cells are detected in gastric contents as hydrochloric acid. The hydrogen ions maintain the acid pH of the gastric contents in which proteins are digested by pepsin. The difference in the average chloride ion concentration in the gastric contents between uninfected and infected abomasa was not significant, even in severe terminal infections where the pH of the gastric contents approached 7.0. Therefore, chloride ions were available in the gastric contents from both uninfected and infected calves to repel the larvae of *O. ostertagi* in vivo (Table 1).

Rutherford and Croll (1979) reported that sodium chloride at different concentrations both attracted and repelled *Caenorhabditis elegans*. The present data

partially supported those conclusions. Neither sodium chloride nor gastric contents repelled exsheathed larvae of *O. ostertagi* when they were diluted to less than 0.5 mEq Cl⁻/liter (Figure 2). This observation might be interpreted as attraction. As stated above, the response curves for both sodium chloride and gastric contents were not identical when superimposed on one another (Figs. 2, 3). The difference probably reflected the different diffusion rate of each repellent. The reason for the different rates of diffusion was undetermined. An additional factor might be present in gastric contents that facilitated its faster diffusion rate. Nevertheless, these data suggested that the chloride ion was the repellent in the gastric contents, because equal concentrations of chloride ion from both sodium chloride and gastric contents gave similar results when tested under different experimental conditions.

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Increased Charges and Dues

As announced in the Proceedings, 49(2):188, 1982, increasing costs have necessitated increases in all charges. In 1983 (Volume 50): domestic subscriptions are \$22, foreign \$24; page charges for authors are raised from \$20 to \$30 per page. Annual membership dues in the Society, *The Helminthological Society of Washington*, are \$15. We regret the error of continuing the prior rates on the cover of this year's January issue (Volume 50, No. 1, 1983). Effective in 1984 (Volume 51): domestic subscriptions will be \$30, foreign \$33. Membership dues and page charges will be unchanged. **Membership dues for 1984, if paid after December 31, 1983, must be increased by a \$3 late charge to cover extra handling costs.**

EDITOR

Helminth Parasites of the Gastrointestinal Tracts and Lungs of Moose (*Alces alces*) and Wapiti (*Cervus elaphus*) from Cypress Hills, Alberta, Canada

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ABSTRACT: Gastrointestinal tracts and lungs of 140 moose (*Alces alces*) and 186 wapiti (*Cervus elaphus*) from Cypress Hills, Alberta, were examined for helminth parasites. Moose were infected with nine species of parasites, including six nematodes, two cestodes, and one trematode. Wapiti were infected with 10 species, including eight nematodes and two cestodes. Six of the total of 13 species found co-occurred in both wapiti and moose. An anoplocephalid tapeworm, *Thysanosoma actinioides*, was the most prevalent parasite in both hosts, occurring in 70% of moose and 56% of wapiti. Other parasites that co-occurred included *Trichuris* sp., *Dictyocaulus viviparus*, *Nematodirella alcidis*, *Orthostromylus macrotis*, and *Taenia hydatigena*.

The Cypress Hills area is a plateau in the southeastern corner of Alberta that crosses the Saskatchewan border (49°30'N, 110°0'W) and comprises an area of approximately 531,000 ha. The hills range in elevation from 1,466 m at their western end in Alberta to 1,067 m in Saskatchewan. The area represents an island habitat of quaking aspen (*Populus tremuloides*), balsam poplar (*Populus balsamifera*), lodgepole pine (*Pinus contorta*), and white spruce (*Picea glauca*) forests surrounded by and interdigitated with prairie grassland (for details see Newsome and Dix, 1968).

Cervids, represented by moose (*Alces alces*), wapiti (*Cervus elaphus*), white-tailed deer (*Odocoileus virginianus*), and mule deer (*O. hemionus*), are common. Several studies have been conducted on parasites of moose and wapiti in Alberta (Stelfox, 1962; Flook and Stenton, 1969; Samuel, 1972a, b), but these have either presented information on a particular parasite(s) or have provided only anecdotal, mainly qualitative, data. Samuel et al. (1976) presented quantitative parasite data on moose in Alberta, including 24 samples from Cypress Hills.

During a herd reduction program in 1978, the gastrointestinal tracts and lungs of 140 moose and 186 wapiti were collected and later examined for helminth parasites. This permitted us to (1) provide basic quantitative data on parasites of sympatric populations of moose and wapiti; (2) compare the parasite faunas of hosts from this unique habitat with those reported from other parts of Alberta and North America; and (3) compare and contrast the findings of this study with those of Samuel et al. (1976) for moose that had been collected in 1970 from the Cypress Hills, in order to discern any major changes that might have occurred over a span of 8 yr.

Materials and Methods

Gastrointestinal tracts and lungs of hunter-killed moose and wapiti were collected in October, November, and December 1978. Samples were frozen on the day of collection for examination later. The contents of lungs, abomasa, the anterior 1.5 m of small intestine (Samuel et al., 1976), and intestinal material adhering to the cecal wall were flushed with tap water into pails. The material

was frothed with tap water and allowed to settle for 3–5 min; the upper contents of the pails were then decanted. Suspended and settled material was refrothed and decanted until samples were clear for visual analysis using a glass tray on a fluorescent-light table. If parasites were found during the initial inspection of the glass tray, the sample was reanalyzed until all parasites had been collected. If no parasites were found during the initial inspection, the tray was reanalyzed.

Approximately 100 cm² of mucosal scrapings from each the abomasum, small intestine, and cecum were examined under a dissecting microscope (0.7–3×). In addition, trachea and bronchioles were opened longitudinally and examined visually for parasites adhering to the inner wall surfaces after flushing. Cestodes and trematodes were preserved in acetic acid-formalin-alcohol (AFA), nematodes in glycerin-alcohol. Representative specimens were deposited in the Canadian National Parasite Collection, Ottawa, Ontario K1A 0M8, accession numbers NMIC(P) 1981-496 to NMIC(P) 1981-500, and NMIC(P) 1981-504 to NMIC(P) 1981-517. Quantitative ecological and epidemiological terminology follows recommendations of the Canadian Society of Zoology.

Results

Parasites of moose (*Alces alces*)

In total, nine parasites were found, including six nematodes, two cestodes, and one trematode (Table 1). The most prevalent parasite was the anoplocephalid cestode *Thysanosoma actinioides*, which occurred in 70% of the moose. The majority of the tapeworms were located in the anterior small intestine; however, in 63% of the cases *T. actinioides* were also found in the abomasa. Other parasites that occurred in more than 10% of the moose included the lungworms *Dictyocaulus viviparus* (17%) and *Orthostrongylus macrotis* (17%). The lungworms co-occurred in only one infection, which suggested a possible negative interaction; however, a χ^2 test failed to reveal a significant negative association ($\chi^2 = 3.42$, $P < 0.10$). The whipworm *Trichuris* sp. (34%) and two trichostrongylid nematodes, *Nematodirella alcidis* (52%) and *Ostertagia* sp. (14%), were also present in more than 10% of the moose. Intensities of parasites were generally low; however, two parasites (*N. alcidis* and *Zygocotyle lunata*) were represented in some infections by more than 100 individuals.

Parasites of wapiti (*Cervus elaphus*)

Ten species of parasites were found, including eight nematodes and two cestodes (Table 1). As in moose, the most prevalent parasite was *Thysanosoma actinioides*, which occurred in the anterior small intestines and abomasa of 56% of the wapiti. Of the remaining species, only *D. viviparus* and *Trichuris* sp. occurred in more than 10% of the wapiti. Although the other seven parasites were not common, two species were present in high-intensity infections (164 *Nematodirus helvetianus* and 84 *Nematodirella alcidis*).

Discussion

Thysanosoma actinioides has been reported from wapiti several times previously, ranging in prevalence from 8% to 50% (D. M. Hammond, unpubl., in Allen, 1973; and C. N. Winger, unpubl., in Honess and Winter, 1956; respectively).

Table 1. Prevalence and intensity of gastrointestinal and pulmonary helminth parasites of moose (*Alces alces*) and wapiti (*Cervus elaphus*) from Cypress Hills, Alberta, 1978.

	Site*	Moose (N = 140)			Wapiti (N = 186)		
		Prev.	Intensity	Range	Prev.	Intensity	Range
Cestodes							
<i>Taenia hydatigena</i> (Pallas, 1766)	ME	5	3	1-4	5	6	1-12
<i>Thysanosoma actinioides</i> Diesing, 1834	A, SI	70	8	1-48	56	9	1-44
Trematodes							
<i>Zygocotyle lunata</i> (Diesing, 1835)	CM	7	42	3-120			
Nematodes							
<i>Cooperia oncophora</i> (Railliet, 1898)	SI				1	21	
<i>Dictyocaulus viviparus</i> (Bloch, 1795)	LU	17	5	1-22	54	10	1-54
<i>Nematodirella alcidis</i> (Dikmans, 1935)	SI	52	38	1-250	1	84	
<i>Nematodirus helvetianus</i> May, 1920	SI				9	43	1-164
<i>Orthostrongylus macrotis</i> (Dikmans, 1931)	LU	17	6	1-15	2	4	1-7
<i>Ostertagia</i> sp.†	A	14	1	1-3			
<i>Skrjabinagia</i> sp.‡					1	2	
<i>Trichostrongylus axei</i> (Cobbold, 1879)	A,SI				5	3	1-4
<i>Trichostrongylus longispicularis</i> Gorden, 1933	SI	2	4				
<i>Trichuris</i> sp.†	CM	34	3	1-17	20	5	1-13

* Sites: A = abomasum. CM = cecum. LU = lungs. ME = mesenteries. SI = small intestine.

† Only females found.

‡ A single male found with spicules unlike any member of the genus.

Allen (1973) indicated that prevalences of 41% for *T. actinioides* in wapiti in Yellowstone Park, Wyoming (Jacobson et al., 1969), and 33% in the Canadian Rockies (Flook and Stenton, 1969) were comparable to prevalences in domestic sheep, whereas deer, regardless of species, were not as suitable hosts as "... other wild ruminants and domestic sheep."

Prevalence of *T. actinioides* in moose in this study was significantly higher than the prevalence of infections in moose from Cypress Hills reported by Samuel et al. (1976) ($\chi^2 = 5.40$, $P < 0.05$) or infections in wapiti in our study ($\chi^2 = 6.40$, $P < 0.05$). This suggests that moose were the most heavily utilized sylvatic host in the Cypress Hills and that the levels of infection had risen significantly in an 8-yr period.

Although the prevalences of *T. actinioides* in both moose and wapiti were the highest yet reported for both hosts in North America, the impact of the parasite on host populations is probably negligible. Allen (1973) stated that despite gross pathological changes in the biliary system and duodenum, the parasite probably causes little clinical disease.

The distribution of *T. actinioides* in both wapiti and moose was unusual because worms were often found in the abomasa. The fringed tapeworm is generally considered to be a parasite of the biliary system. The frequency of reports of the parasite in the small intestine, and its occurrence in the abomasa, probably reflect a capacity for rapid postmortem migration, especially as the worms are believed to be very mobile (Allen, 1973).

The second most prevalent parasite of moose in this study was the tricho-

strongylid nematode *Nematodirella alcidis*. There has been some controversy over the taxonomy of this parasite. Dikmans (1935) identified three subspecies of *Nematodirella longispicularis* based on identity of the host, namely, *N. l. antilocaprae* from pronghorn (*Antilocapra americana*), *N. l. longispicularis* from reindeer (*Rangifer tarandus*), and *N. l. alcidis* from moose. Ivashkin (1954) elevated *N. l. alcidis* to the rank of species, *N. alcidis*, based on characteristics of the dorsal ray of the bursa and spicule length. Skrjabin et al. (1954) concurred and provided a key to species of the genus *Nematodirella*. Specimens found in both moose and wapiti in this study could not be differentiated and were identified as *N. alcidis*.

The prevalence of *Nematodirella* in moose did not differ significantly from that reported by Samuel et al. (1976) (52% and 48%, respectively). This indicates that *N. alcidis* is a stable component of the parasite fauna of moose from Cypress Hills. Wapiti were much less utilized hosts for this parasite (1% prevalence, intensity of 84). Another trichostrongylid nematode, *Nematodirus helvetianus*, was found only in wapiti. The genera *Nematodirus* and *Nematodirella* are grouped within the same subfamily, Nematodirinae, thus indicating morphological similarity. This, and the fact that they are both found in the same site in the definitive host, may suggest that they are ecological equivalents. Other trichostrongylid nematodes of the gastrointestinal tract were represented in moose and wapiti by low-prevalence, low-intensity infections.

Two other parasites commonly encountered in moose and wapiti were the lungworms *Dictyocaulus viviparus* and *Orthostrongylus macrotis*. *Dictyocaulus viviparus* has been reported from a wide variety of domestic and wild ruminants (Yamaguti, 1961); *O. macrotis* is a parasite of wild ruminants, and is probably more important in members of the genus *Odocoileus* than in either *Cervus* or *Alces* (see Dougherty and Goble, 1946).

Dictyocaulus viviparus was the second most prevalent parasite in wapiti in this study (54%). Other reports of *D. viviparus* in wapiti revealed prevalences ranging from 90% in the Athabasca Valley of Banff Park, Alberta (Cowan, 1951), to 4%, also in the Canadian Rockies (Flook and Stenton, 1969). Other studies reported prevalences of 8% in Montana (Barrett and Worley, 1966), 10% in South Dakota (Boddicker and Huggins, 1969), 23% in New Mexico (Wilson, 1969), 35% for calves and 44% for adults from western Montana (Worley et al., 1969), and 60% in Wyoming (Bergstrom, 1975). Therefore, prevalences of *D. viviparus* may vary widely, even within the same geographic regions at different times of the year. The prevalence of *D. viviparus* was significantly higher in wapiti than in moose ($\chi^2 = 45.22$, $P < 0.001$). *Orthostrongylus macrotis*, however, was significantly more prevalent in moose than in wapiti ($\chi^2 = 22.86$, $P < 0.001$). These data, in conjunction with the reported absence of any lungworms in moose from the Cypress Hills by Samuel et al. (1976), may indicate an increased overlap in range utilization by ruminants, both wild and domestic, in the Cypress Hills. Presidente et al. (1972) and Presidente and Knapp (1973) showed that *D. viviparus* strains from domestic animals were more infective to wild ruminants (*C. elaphus* and *O. hemionus*) than strains from wild ruminants were to domestics. Gupta and Gibbs (1971) found similar results with moose-strain *D. viviparus*. Therefore, because food habits of wapiti, cattle, and sheep using the same range can be very similar (Stevens, 1966), acquisition of direct-life-cycle nematodes by wapiti could be anticipated.

Another parasite that may indicate interaction between sylvatic and domestic animals was the cecal whipworm *Trichuris ovis*. This is a generalist parasite found in many herbivorous hosts (Skrjabin et al., 1957). Although *Trichuris* was found in 34% of moose and 20% of wapiti in this study, with moderate intensities (up to 17 in moose), no male worms were found. Sizes of worms and characteristics of the uteri and vulvae of females closely resembled those described for *T. ovis* (Knight, 1971). Thus, the worms are tentatively called *T. ovis*. Samuel et al. (1976) found 14% of moose in the Cypress Hills infected.

Although all other parasites were found in low prevalences, one is noteworthy because of an intense infection (120 worms) in a moose. *Zygocotyle lunata* is an amphistome trematode of waterfowl, however ungulates have previously been reported as hosts (Swanson, 1959; Samuel et al., 1976). Samuel et al. (1976) did not find infected moose in the Cypress Hills, "where relatively few potholes and waterfowl occur in moose habitats."

Overall, the parasite faunas of moose and wapiti from the Cypress Hills were similar; the hosts shared six of a total of 13 parasites. The most prevalent parasite in both hosts was *T. actinioides*, and the faunas of both were dominated by species of trichostrongylid nematodes.

The composition of the parasite faunas of both hosts did not differ greatly from those in other regions of Alberta or other geographic areas. Often, however, prevalences for individual species of helminths were as high as, or higher than, any reported previously.

A comparison of this work with Samuel et al. (1976) demonstrated several changes in the parasite fauna of moose of the Cypress Hills over an 8-yr period. Three additional species of parasites were found, including two species of lungworms, whereas a cestode, *Moniezia* sp., found in 1970 was not present in moose in this survey. The additional species found in our study may simply reflect the difference in sample size between the two surveys (140 vs. 24), but the higher prevalences of most species and the disappearance of *Moniezia* sp. argue for real changes. Taken in its entirety, the evidence suggests a pattern of extensive range overlap among moose, wapiti, and domestic animals.

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Helminths of Black Bears in Florida¹

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ABSTRACT: Examination of 46 black bears (*Ursus americanus*) collected in Florida over a 5-yr period revealed 14 species of helminth parasites: 12 nematodes, one trematode, and one acanthocephalan. The mean number of parasite species per bear was four (range 1-8), and no significant differences ($P < 0.05$) were observed among sexes and ages. The mean intensity was 406 worms per infected bear (range 1-4,929), and although no significant difference was found among ages, mean intensities were significantly higher in male bears. The three most prevalent parasites were *Strongyloides* sp. (87%), *Macracanthorhynchus ingens* (80%), and *Capillaria aerophila* (73%). *Strongyloides* sp. was the dominant helminth in terms of prevalence and intensity. There were no significant differences in the prevalences or intensities of infection of individual helminth species in reference to age, but there was a greater prevalence and intensity of *C. aerophila*, and a greater intensity of *M. ingens* in male bears.

Early settlers viewed the omnivorous black bear as a competitor for food and space, and this resulted in the indiscriminate killing of the species for many generations (Jonkel, 1978). Today, new pressures such as increasing human populations and habitat destruction have brought about a decline in the numbers of black bears, in addition to causing scattered populations. This is especially true of black bears in the eastern United States (Jonkel, 1978). With this in mind, the role of potential disease agents in isolated populations of black bears becomes extremely important. Efforts to determine the potential pathogens of black bears must be given full consideration in order to help conserve this species.

There have been several recent reports on the endoparasites of black bears from different regions in North America. A review of the available literature from the United States and Canada can be found in Pence et al. (1982). In the southeastern U.S., a study by Crum et al. (1978) on the helminths of black bears was prompted due to the paucity of information from this region. In their study, only four bears from Florida were examined. We collected helminths from 46 black bears over a 5-yr period to help understand the nature of parasitism in bears from Florida, and a summary of these data is presented herein.

Materials and Methods

From October 1976 through April 1981, 46 black bears were collected from 11 localities in Florida (Fig. 1). Most of these ($N = 33$) were hunter-killed animals from Osceola National Forest in northern Florida (Baker and Columbia counties); the remainder consisted of road-killed bears from scattered locations throughout the state. Consequently, the majority of the bears was collected during the hunting season (October through December), with occasional bears taken at other times. The age of each animal was determined by cementum analysis of tooth sections

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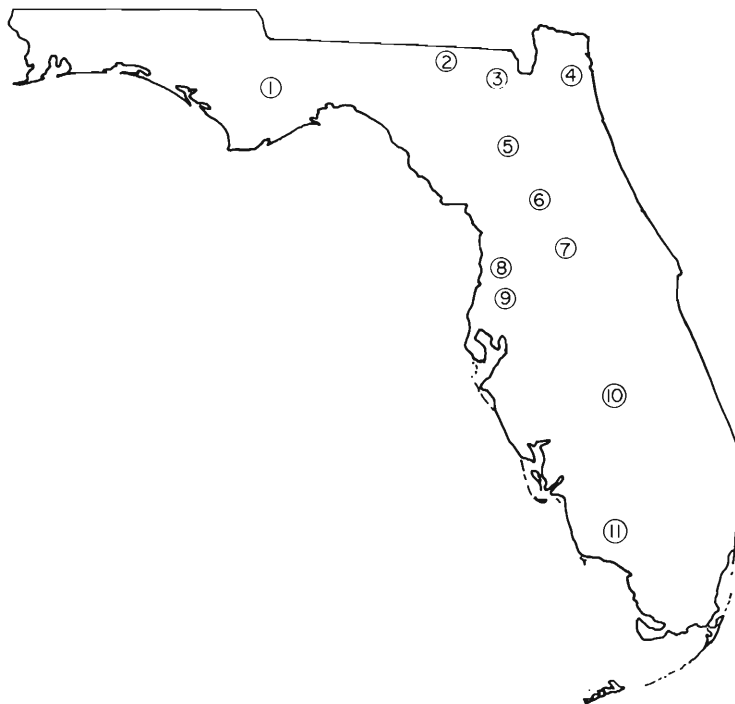


Figure 1. Collection sites of black bears in Florida. Numbers of bears collected from each site are as follows: (1) Liberty County ($N = 2$); (2) Hamilton County ($N = 1$); (3) Columbia and Baker counties ($N = 33$); (4) Duval County ($N = 2$); (5) Alachua County ($N = 1$); (6) Marion County ($N = 1$); (7) Lake County ($N = 1$); (8) Hernando County ($N = 1$); (9) Pasco County ($N = 1$); (10) Highlands County ($N = 1$); (11) Collier County ($N = 1$). (Location was not available for one bear.)

on the first premolar (Willey, 1974). Viscera were removed in the field and frozen until they could be analyzed. Sample sizes differed for some parasite species due to the unavailability of certain organs. We followed techniques for the recovery of helminths as described by Kinsella and Forrester (1972), including the use of fine-mesh screens. In addition, we checked for larvae of *Trichinella* sp. in the diaphragms of 25 bears by tissue squash and/or pepsin-HCl digestion. Representative helminths were deposited in the U.S. National Parasite Collection in Beltsville, Maryland (accession numbers in Table 1).

Chi-square and t tests ($P < 0.05$) were performed on the prevalences and logarithmically transformed intensities of infection, respectively, with regard to sex and age (Steel and Torrie, 1980).

Results and Discussion

Table 1 shows the species of helminths recovered, locations within the viscera, and prevalences and intensities of infection of each parasite. There were 12 nematode, one trematode, and one acanthocephalan species. The three most prevalent helminths were *Strongyloides* sp. (87%), *Macracanthorhynchus ingens* (80%), and *Capillaria aerophila* (73%). Not only was *Strongyloides* sp. the most prevalent parasite, it also had the greatest mean intensity ($\bar{x} = 412$). Of 14,204 helminths

Table 1. Location, prevalence, and intensity of infection of helminth parasites of black bears in Florida, 1976–1981.

Parasite	USNM no.	Sample size*	Prevalence	Intensity†			
				Mean	Median	Range	
Acanthocephala							
<i>Macracanthorhyncus ingens</i> (von Linstow, 1879) Meyer, 1933 (1)‡	77033	46	80.4	8.8	6.0	1–46	
Nematoda							
<i>Strongyloides</i> sp. (1)	77034	46	87.0	412.0§	30.5	1–4,767	
<i>Capillaria aerophila</i> (Creplin, 1839) Travassos, 1915 (2)	77035	41	73.2	59.5	34.0	4–367	
<i>Molineus barbatus</i> Chandler, 1942 (1)	77036	46	45.7	10.0§	4.5	1–79	
<i>Gongylonema pulchrum</i> Molin, 1857 (3)	77037	34	38.2	2.4	1.0	1–9	
<i>Placoconus lotoris</i> (Schwartz, 1925) Chandler, 1942 (1)	77038	46	28.3	4.1§	3.0	1–11	
<i>Gnathostoma</i> sp. (1, 3, 4, 5, 6)	77039	46	17.4	1.5	1.0	1–3	
<i>Physaloptera</i> sp. (6)	77040	46	15.2	2.1	1.0	1–5	
<i>Dirofilaria immitis</i> (Leidy, 1856) Railliet and Henry, 1911 (2, 7)¶	77041	40	15.0	1.2	1.0	1–2	
<i>Capillaria putorii</i> (Rudolphi, 1819) Travassos, 1915 (6)	77043	46	8.7	3.0	3.0	1–5	
<i>Gnathostoma didelphis</i> Chan- dler, 1932 (3, 4, 9)	77171	46	6.5	1.3	1.0	1–2	
<i>Capillaria plica</i> (Rudolphi, 1819) Travassos, 1915 (8)	77044	20	5.0	15.0	15.0	15	
<i>Lagochilascaris</i> sp. (6)#	77042	46	4.3	63.5	63.5	24–103	
<i>Toxascaris leonina</i> (von Lin- stow, 1902) Leiper, 1907 (2)	77279	46	4.3	2.5	2.5	1–4	
<i>Physaloptera rara</i> Hall and Wigdor, 1918 (6)	77173	46	4.3	10.5	10.5	1–20	
<i>Crenosoma vulpis</i> (Dujardin, 1844) Railliet, 1915 (2)	77172	41	2.4	1.0	1.0	1	
<i>Crenosoma</i> sp. (2)**	77045	41	2.4	1.0	1.0	1	
Trematoda							
<i>Pharyngostomoides procyonis</i> Harkema, 1942 (1)	77046	46	2.2	1.0§	1.0	1	

* Sample sizes differ due to unavailability of organs from some bears.

† Number of worms/infected bear.

‡ Numbers in parentheses indicate site in host: (1) intestine; (2) lungs, trachea; (3) esophagus; (4) liver; (5) spleen; (6) stomach; (7) heart; (8) urinary bladder; (9) pancreas.

§ Intensities for these species are based on results from 35 bears only.

|| Larval stages.

¶ No microfilariae in adult females.

One bear had 103 immature worms in a purulent abscess; another had mature worms of an undescribed species.

** Close to *Crenosoma goblei* Dougherty, 1945.

recovered from 35 bears, 87% were *Strongyloides* sp., followed by *C. aerophila* (9%) and *M. ingens* (2%). All bears were infected with at least one species of parasite, with an average of four species per bear (range 1–8). The number of species per bear and prevalences out of bears infected with that number of species

Table 2. Ranges and means of the number of parasite species and intensities with regard to sex and age.

Category	Number of parasite species			Intensity		
	N	Range	Mean	N	Range	Mean
Sex						
Males	27	1-8	4.4a*	21	2-4,929	554.6b
Females	19	1-8	3.7a	14	1-2,197	182.6c
Age-class						
2-4 years	16	1-7	4.5d	13	15-4,929	538.2e
>4 years	16	1-8	4.2d	11	2-2,197	464.4e

* Mean values followed by similar letters are not significantly different; values followed by dissimilar letter are significantly different.

were as follows: one (13%), two (11%), three (20%), four (15%), five (9%), six (22%), seven (6%), and eight (4%).

Table 2 shows the ranges and means for the number of parasite species, and intensity of infection for sex and age. There were no significant differences in the mean numbers of parasite species per bear among sex and age categories. Based upon the 35 bears for which total counts were obtained, intensities ranged from one to 4,929 (\bar{x} = 406). Although there was no significant difference in the mean intensity among ages, a significant difference was found between sexes, with male bears exhibiting higher mean intensities than females.

With regard to sex, there was a significantly greater prevalence of *C. aerophila* in males when all bears were considered, but no significant difference in bears from Osceola only (N = 33). *Physaloptera* was found in only eight male bears. The intensities of *C. aerophila* and *M. ingens* were significantly greater in males for both the total and Osceola samples of bears. These findings, in addition to the greater intensity of infection found in male bears when all helminths are considered, may be related to the much larger home range sizes of males as compared to female bears (Garshelis and Pelton, 1981). The increased mobility of male bears may result in greater sources for infection, especially if there is more overlap in home range than is exhibited by females. With regard to age, no significant differences in the prevalences or intensities of infection were found for any species of helminth analyzed.

New host records were established with the finding of *Gnathostoma didelphis*, *Capillaria plica*, *Lagochilascaris* sp., and *Pharyngostomoides procyonis*. The remainder of the helminths recovered in this study have been reported previously from black bears, and many are shared with the raccoon (*Procyon lotor*) owing to the close taxonomic relationship between these two mammals (Pence et al., 1982). In general our study on 46 bears in Florida, most of which were from one area, agreed with data reported by Crum et al. (1978) from smaller sample sizes in a variety of southeastern states. As in their study, larvae of *Trichinella* sp. were not recovered. Notably different were the much greater prevalences of *M. ingens*, *Strongyloides* sp., and *C. aerophila*, and the greater mean intensity of *Strongyloides* sp. in our study. These observations may indicate that there is a large degree of interaction between bears due to high population densities and/or overlap of home ranges.

The pathological impact of the helminths recovered is largely unknown, although Crum et al. (1978) reported that *C. aerophila* in high numbers was associated with catarrhal bronchitis. The large size and powerful hooks on the proboscis of *M. ingens* undoubtedly cause serious pathological changes in the wall of the intestine (Petrochenko, 1971). Because several species of *Strongyloides* are known to be pathogenic to young animals (Levine, 1980), the high prevalence and intensity of this parasite in black bears in Florida is also of concern. Further studies are needed to understand the significance of *C. aerophila*, *M. ingens*, and *Strongyloides* sp. infections, especially in bears under 2 yr of age, which were not examined in the present study.

Acknowledgments

Appreciation is extended to David S. Maehr and other members of the Florida Game and Fresh Water Fish Commission for help in obtaining the bears for this study. In addition, the many bear hunters in Florida should be thanked for their cooperation in this effort. Thanks are due Garry W. Foster and Pamela P. Humphrey for technical assistance in the necropsies of many of the bears, and to R. Kipp Frohlich for assistance with the data analysis.

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A Comparison of the Helminth Fauna of Two *Plethodon jordani* Populations from Different Altitudes in North Carolina

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ABSTRACT: Of 195 *Plethodon jordani* collected in Jackson County, North Carolina, at elevations of 914 m and 1,170 m, 96 (49.2%) were infected with *Cosmocercoides dukae*, 14 (7.2%) with *Cylindrotaenia americana*, three (1.5%) with *Brachycoelium salamandrae*, one (0.5%) with an acanthocephalan, and 17 (8.7%) with nematode larvae encysted in the coelom. There was no difference in diversity, prevalence, or intensity at the two altitudes ($P > 0.005$). *Cosmocercoides dukae* and *Cylindrotaenia americana* have not been reported previously from *P. jordani*.

Although the biology and habitats of the Appalachian woodland salamander (*Plethodon jordani*) have been investigated, little information is available on its helminth fauna, and because this species is for the most part a high-altitude form, extending upward to 2,000 m, even less is known concerning the diversity and prevalence of helminth infections at various elevations. This report presents a comparison of the diversity, prevalence, and intensity of *P. jordani* helminths collected at two different elevations within the same geographic area. In addition, parasitological findings in this investigation are compared with those reported from other terrestrial species of salamanders.

Materials and Methods

Salamanders were sampled from two different altitudes in Jackson County, North Carolina. Eighty-five specimens were taken at an elevation of 914 m and 110 specimens at an elevation of 1,170 m. Trematodes, cestodes, and the single acanthocephalan specimen were fixed in alcohol–formalin–acetic acid (AFA), stained in Harris' hematoxylin, cleared in beechwood creosote, and mounted in Canada balsam. Nematodes were fixed in hot ethanol, cleared in glycerin, and studied as temporary mounts.

Statistical tests were performed on an IBM Model 370/158 computer using SAS (Statistical Analysis System).

Results

Of the 195 *Plethodon jordani* examined from both altitudes, 114 (58.5%) were infected. This included 96 (49.2%) infected with *Cosmocercoides dukae* (Holl, 1928), 14 (7.2%) with *Cylindrotaenia americana* Jewell, 1916, three (1.5%) with *Brachycoelium salamandrae* (Froelich, 1789), one (0.5%) with an acanthocephalan, and 17 (8.7%) with nematode larvae encysted in the coelom (Table 1). Eighty-one (41.5%) were free of helminths. Ninety-eight *P. jordani* harbored only a single species, two species were found in 15 animals, and three in one. The number of specimens of each helminth per host ranged from two to 28 for *B. salamandrae*, one to eight for *C. dukae*, one to three for *C. americana*, and three to five for the encysted nematode larvae.

Representative specimens of *B. salamandrae*, *C. dukae*, and *C. americana* are

Table 1. Prevalence of single, multiple, and combined infections of *Plethodon jordani* from two elevations in Jackson County, North Carolina.

Parasite	Elevation	
	914 m	1,170 m
	Number (%)	Number (%)
Single infections		
<i>Cosmocercoides dukae</i>	34 (40.0)	46 (41.8)
<i>Cylindrotaenia americana</i>	5 (5.9)	5 (4.5)
<i>Brachycoelium salamandrae</i>	0	0
Acanthocephalans	1 (1.2)	0
Encysted nematode larvae	4 (4.7)	3 (2.7)
Multiple infections		
<i>C.d.</i> + <i>C.a.</i>	1 (1.2)	2 (1.8)
<i>C.d.</i> + e.n.l.	4 (4.7)	5 (4.5)
<i>C.d.</i> + <i>C.a.</i> + e.n.l.	1 (1.2)	0
<i>C.d.</i> + <i>B.s.</i>	1 (1.2)	2 (1.8)
Combined (single and multiple) infections		
<i>Cosmocercoides dukae</i>	41 (48.2)	55 (50)
<i>Cylindrotaenia americana</i>	7 (8.2)	7 (6.4)
<i>Brachycoelium salamandrae</i>	1 (1.2)	2 (1.8)
Acanthocephalans	1 (1.2)	0
Encysted nematode larvae	9 (10.6)	8 (7.3)

deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, and assigned numbers 77085, 77083 and 77086, respectively.

Discussion

Species of *Brachycoelium* are the most common flukes encountered in salamanders, and in the present study *B. salamandrae* was the only fluke encountered. It has been reported from several species of salamanders in North Carolina, including *Ambystoma maculatum*, *Ambystoma opacum*, *Desmognathus fuscus*, *Desmognathus ochrophaeus carolinensis*, *Desmognathus monticola*, *Desmognathus quadramaculatus*, *Eurycea b. cirrigera*, *Eurycea b. wilderae*, *Eurycea longicauda guttolineata*, *Notophthalmus viridescens*, *Plethodon cinereus*, *Plethodon glutinosus*, *Plethodon j. jordani*, *Plethodon j. metcalfi*, *Plethodon yonahlossee*, and *Pseudotriton ruber* (see Mann, 1932; Rankin, 1937, 1938; Russell, 1951).

That the prevalence of *B. salamandrae* in *P. jordani* at both altitudes is not significantly different concurs with the findings of Powders (1968), who determined that the prevalence of *Brachycoelium* in *P. jordani* from Tennessee was independent of elevation.

Cosmocercoides dukae has been recorded from many terrestrial and aquatic amphibians in the contiguous United States and Canada. Salamanders in North Carolina implicated as hosts include *Ambystoma opacum*, *Desmognathus fuscus*, *Notophthalmus viridescens*, *Plethodon cinereus*, *Plethodon glutinosus*, and *Plethodon longicrus* (see Holl, 1928, 1932; Harwood, 1932; Mann, 1932; Rankin, 1937; McGraw, 1968). Its occurrence in *Plethodon jordani* from North Carolina constitutes a new host record.

Several authors have reported life-history studies on this parasite. Anderson (1960) described the development and transmission of *C. dukae* in terrestrial molluscs. Anderson (1960) and Ogren (1953) demonstrated that third-stage larvae enter the respiratory pore of snails and develop in the mantle cavity. In addition, Anderson (1960) described transovarial infection in snails. McGraw (1968) showed that snails can also become infected by ingesting contaminated amphibian feces. Baker (1978) reported transmission of third-stage larvae of *C. dukae*, obtained from cultures of feces from wild *Bufo americanus* harboring the parasite, to parasite-free *B. americanus* by skin penetration. With the demonstration of apparently independent life cycles of *C. dukae* in molluscs and amphibians and the transfer of this nematode between the two groups, it can be readily understood why *C. dukae* in the present investigation was the most frequently encountered helminth at both altitudes.

Cylindrotaenia americana was the only tapeworm detected in this study. Originally described by Jewell (1916) from the intestine of various anurans including the southern cricket frog (*Acris gryllus*), it has been reported in *Desmognathus f. fuscus* from North Carolina by Mann (1932). Its occurrence in *P. jordani* constitutes a new host record.

With the exception of Brandt (1936), who in a survey of 368 amphibians of North Carolina observed tapeworm infections ranging from zero to 51% in six species of hosts, extensive surveys of helminth parasites of amphibians have revealed low prevalences of cestodes.

Few species of acanthocephalans have been reported from salamanders in North America. With the exception of the report by Nickol (1969) of 10 of 11 *Necturus beyeri* from Louisiana found infected with *Fessesentis necturorum* Nickol, 1967, the prevalence of acanthocephalan infections in salamanders as reported by other investigators is low. Only a single acanthocephalan specimen that could not be identified was found in the present study.

The diversity and prevalence of helminths in *Plethodon jordani* correlate with those reported from closely related species of terrestrial salamanders. Fischthal (1955) detected few parasites and low prevalences in 25 *Plethodon g. glutinosus* and 36 *Plethodon c. cinereus* examined from south-central New York. Fischthal also found multiple infections to be rare, with none occurring in *P. c. cinereus* and only one with two species of parasites in *P. g. glutinosus*. Rankin (1937), who examined over a thousand salamanders representing 19 species from North Carolina, concluded that terrestrial salamanders harbor few parasites, and those in low numbers.

It is unfortunate that in the present study salamanders were available from only two altitudes. Information on parasitism in *P. jordani* from other elevations might prove valuable in ascertaining the altitudinal limits of the helminths recovered.

Acknowledgments

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Cuticular Ridge Patterns of *Nematodirus* (Nematoda: Trichostrongyloidea) Parasitic in Domestic Ruminants of North America, with a Key to Species

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ABSTRACT: The six species of *Nematodirus* parasitic in domestic ruminants of North America have been identified previously on the basis of characteristics of the bursa and tips of the spicules, and females could not be identified. In an effort to find additional diagnostic characteristics of both sexes, cuticular ridges were studied with light and scanning electron microscopy and in whole mounts and cross sections. After the cuticular ridges of males were characterized, females were matched with males by means of cuticular ridges, except for the rare species *N. davtiani*. Five of the six species have variations of an 18-ridge bilaterally symmetrical system in the cervical region. The sixth species has 26 cervical ridges. Two groups of species were recognized on the basis of cuticular characteristics correlated with other morphological characters. The two species in Group I, *Nematodirus filicollis* and *N. davtiani*, lose ridges laterally in the postcervical region and have 14 ridges at midbody. They can be identified by their anteriorly extended pattern of ridges in the cervical region. These two species also share the characteristics of finlike ridges, a small number (30–35) of perioral denticles, a short cephalic expansion, and a large bursa without a separate dorsal lobe. *Nematodirus davtiani* can be distinguished from *N. filicollis* by its prominent dorsalmost and ventralmost ridges and its distinctive dorsal ray. In contrast, the four species of Group II, *N. helvetianus*, *N. oiratianus interruptus* ssp. n., *N. abnormalis*, and *N. spathiger*, share the characteristics of a more posteriorly distributed pattern of ridges in the cervical region, 18 or more ridges near midbody, smaller dorsal and ventral ridges, a larger number (50–65) of perioral denticles, a longer cephalic expansion, and a smaller bursa with separate dorsal lobes. *Nematodirus helvetianus* and *N. oiratianus interruptus* add ridges in the cervical and postcervical regions, and are characterized by having more than 18 ridges for most of their length; they do not add ventral ridges in the last quarter of the males. *Nematodirus helvetianus* has more ridges (30–36 at midbody) than any of the other species. *Nematodirus oiratianus interruptus* can be easily separated from all other species by its discontinuous ridges in the cervical region. *Nematodirus oiratianus oiratianus* from Asia and South America have continuous ridges. *Nematodirus spathiger* and *N. abnormalis* have 18 ridges for most of their length; they lose all dorsal ridges and add a few ventral ridges in the last quarter of the males. *Nematodirus abnormalis* can be distinguished from *N. spathiger* by the cervical discontinuities in ridges numbered 2 and 8, by spicular and bursal characteristics, and a more anterior vulva position. Possible evolutionary relationships among the six species are described in a cladogram and a key to species.

Trichostrongyloid nematodes are usually identified to species by characteristics of males, and females usually cannot be identified. The six species of *Nematodirus* previously have been identified by characteristics of the spicule tips and copulatory bursae (Becklund and Walker, 1967) and the distribution of bosses on the inside surface of the copulatory bursae (Stringfellow, 1968). Females have been identified only rarely, when they were found in the presence of a single species of male (Herlich, 1954; Biocca et al., 1974).

Recent studies (Lichtenfels, 1971, 1974, 1977; Durette-Desset, 1979; Lichtenfels and Pilitt, 1983) have demonstrated that characteristics of longitudinal ridges on the surface of the cuticle of most trichostrongyloid nematodes can be used to identify both males and females to species. The objective of the present study was to determine whether sufficient differences exist among cuticular ridges of six species of *Nematodirus* parasitic in domestic ruminants of North America (Table 1) to identify the nematodes to species.

Table 1. Specimens of *Nematodirus* spp. studied and illustrated by host and locality.

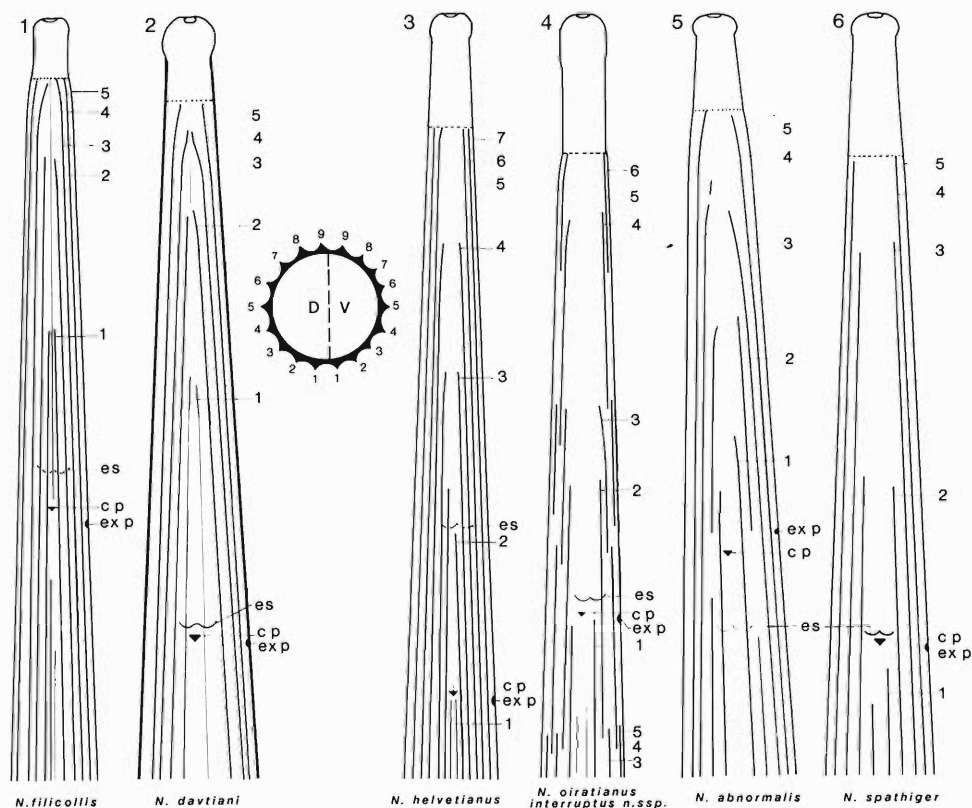
Species and synonyms	Numbers and lots/specimens (Figs.) by host and locality
<i>N. filicollis</i> (Rudolphi, 1802) Ransom, 1907	<i>Ovis aries</i> , sheep
syn.: <i>Ascaris filicollis</i> Rudolphi, 1802	Australia—1/7
<i>Strongylus filicollis</i> (Rud., 1802) Rud., 1803	Colorado, USA—1/1
<i>Fusaria filicollis</i> (Rud., 1802) Zeder, 1803	Maryland, USA—3/11 (19)
<i>Oesophagostomum filicollis</i> (Rud., 1802)	Peru—2/12 (7, 13, 20)
Stossich, 1899	Vermont, USA—2/12 (21–23)
<i>N. furcatus</i> May, 1920 (May, 1924)	Virginia, USA—1/4
	Wyoming, USA—1/3
	<i>Ovis canadensis</i> , bighorn sheep
	Montana, USA—1/2
<i>N. davtiani</i> Grigorian, 1949	<i>Ovis aries</i> , sheep
syn.: <i>N. rufaevastitatis</i> Durbin and Honess, 1951	Wyoming, USA—1/3
(Becklund, 1966)	<i>Ovis ammon</i> , Argali sheep
	British Museum—1/2
	<i>Ovis canadensis</i> , bighorn sheep
	Alberta, Canada—1/1
	Montana, USA—1/3
	<i>Ovis dalli</i> , Dall sheep
	Alaska, USA—2/7 (8, 14, 24–28, 34, 35)
<i>N. helvetianus</i> May, 1920	<i>Bos taurus</i> , cattle
	Alabama, USA—1/5 (32)
	Montana, USA—1/4 (29)
	Wyoming, USA—1/11 (9, 15, 33)
	<i>Ovis canadensis</i> , bighorn sheep
	Montana, USA—1/1 (30, 31)
	<i>Saiga tatarica</i> , saiga gazelle
	Washington, D.C., USA—1/1 (zoo—orig. USSR)
<i>N. oiratianus oiratianus</i> (Raevskaia, 1929)	<i>Gazella subgutterosa</i> , goitered gazelle
syn.: <i>N. oiratianus</i> Raevskaia, 1929	Iran—1/1 (British Museum)
<i>N. lanceolatus</i> Ault, 1944	<i>Ovis aries</i> , sheep
	Argentina—1/1
	Cairo, Egypt—1/6
	Iran—1/3
	Peru—1/3
	<i>Saiga tatarica</i> , saiga gazelle
	Washington, D.C., USA—1/1 (zoo—orig. USSR)
	USSR—1/5
<i>N. oiratianus interruptus</i> ssp. n.	<i>Antilocapra americana</i> , pronghorn antelope
syn.: <i>N. oiratianus</i> Raevskaia, 1929 (only North American records of this species)	New Mexico, USA—1/3
<i>N. lanceolatus</i> Ault, 1944 (only North American records of this species)	<i>Oreamnos americana</i> , mountain goat
	Canada—1/1 (37)
	<i>Ovis aries</i> , sheep
	Alaska, USA—1/2
	Colorado, USA—2/4
	Nebraska, USA—1/2 (53–55)
	New Mexico, USA—2/5 (10, 16)
	Utah, USA—1/2
	Wyoming, USA—1/6

Table 1. Continued.

Species and synonyms	Numbers and lots/specimens (Figs.) by host and locality
	<i>Ovis canadensis</i> , bighorn sheep Montana, USA—7/54 (40, 52) Alberta, Canada—1/2 (38, 39, 41)
	<i>Ovis dalli</i> , Dall sheep Alaska, USA—2/5
<i>N. abnormalis</i> May, 1920	<i>Ovis aries</i> , sheep Australia—2/21 British Columbia, Canada—1/20 (17, 42, 45) Idaho, USA—1/5 (11, 43, 44) Maryland, USA—2/2 Peru—3/11 (46) Utah, USA—1/5 Washington, USA—1/1
	<i>Saiga tatarica</i> , saiga gazelle Washington, D.C., USA—1/1 (zoo—orig. USSR)
<i>N. spathiger</i> (Railliet, 1896) Railliet and Henry, 1909 syn.: <i>Strongylus spathiger</i> Railliet, 1896	<i>Gazella subgutterosa</i> , goitered gazelle Iran—1/2 (British Museum)
	<i>Ovis aries</i> , sheep Australia—1/17 British Columbia, Canada—1/14 (18, 36, 49, 51) Colorado, USA—1/1 Idaho, USA—1/8 Maryland, USA—1/1 Peru—2/9 Utah, USA—2/6 Vermont, USA—2/2 (47) Washington, USA—1/1
	<i>Ovis canadensis</i> , bighorn sheep Montana, USA—2/9 (12, 48, 50)
	<i>Saiga tatarica</i> , saiga gazelle Washington, D.C., USA—1/5 (zoo—orig. USSR)

Materials and Methods

The sources and numbers of specimens studied and the figure numbers in which they are illustrated are listed in Table 1. Whole specimens were studied in temporary mounts cleared in phenol–alcohol (80 parts melted phenol crystals and 20 parts absolute alcohol). Regular light microscopy, interference-contrast light microscopy (Leitz), and scanning electron microscopy were used when sufficient specimens were available. Scanning electron micrographs were obtained by the methods of Madden and Tromba (1976). Cross sections were studied in either free-hand cuts made with a cataract knife, or in paraffin-embedded sections. The cuticular ridges were studied first in males identified by other characteristics. Females were then matched with the males on the basis of characteristics of the cuticular ridges. After females were identified by cuticular characteristics, they



Figures 1-6. *Nematodirus* spp. of North American domesticated ruminants. Diagrammatic drawings of anterior and cervical regions; right lateral views showing cephalic expansions, number and pattern of distribution of cuticular ridges, and positions of cervical papillae (cp), excretory pore (exp), and distal end of esophagus (es). 1. *N. filicollis*. 2. *N. davtiani*. 3. *N. helvetianus*. 4. *N. oiratianus interruptus* ssp. n. 5. *N. abnormalis*. 6. *N. spathiger*.

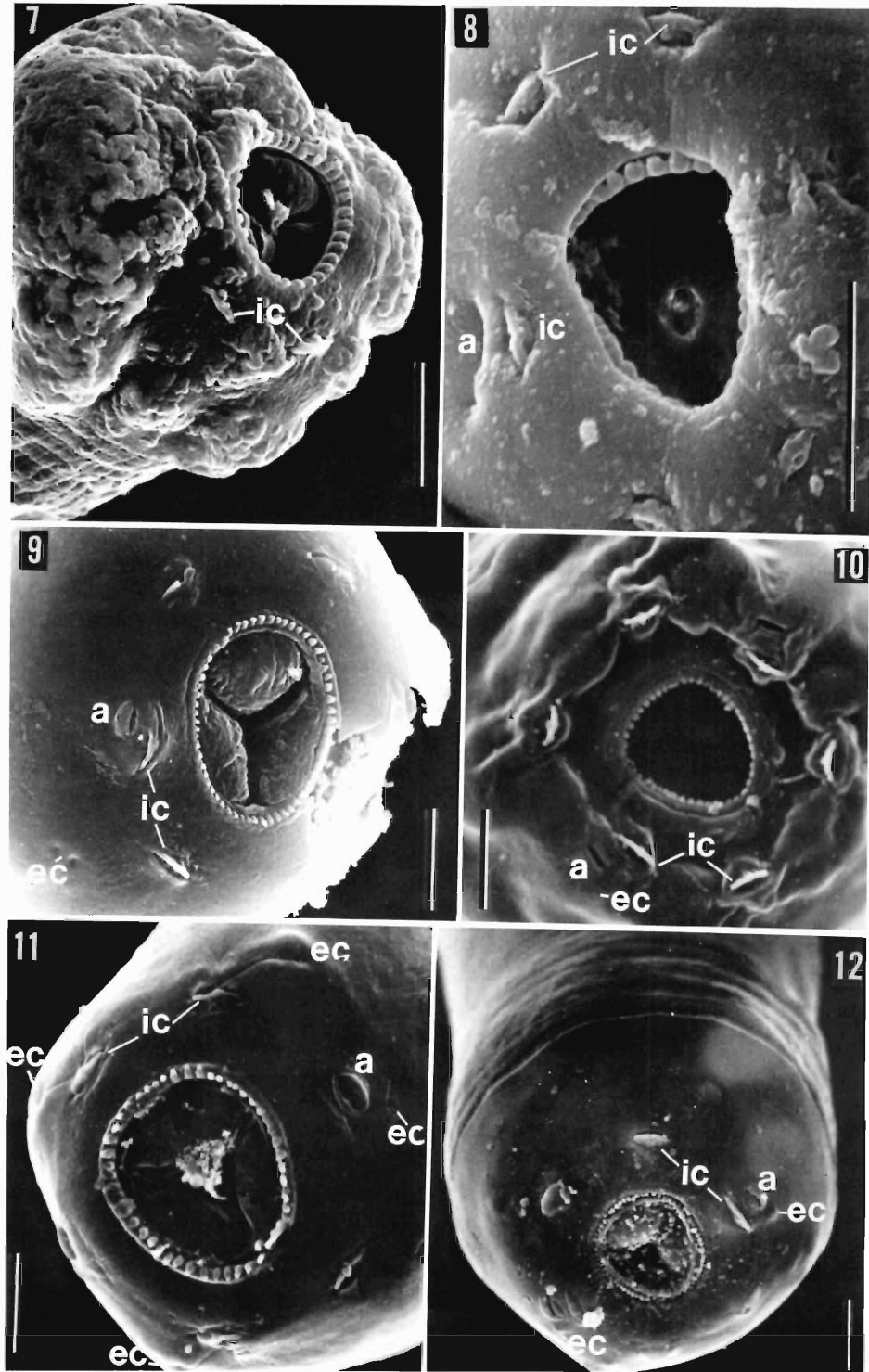
were studied for additional distinguishing characteristics. Measurements are in micrometers unless indicated otherwise. Drawings were made with the aid of a camera lucida. The cladogram and cladistic analysis followed the methods of Hennig (1966).

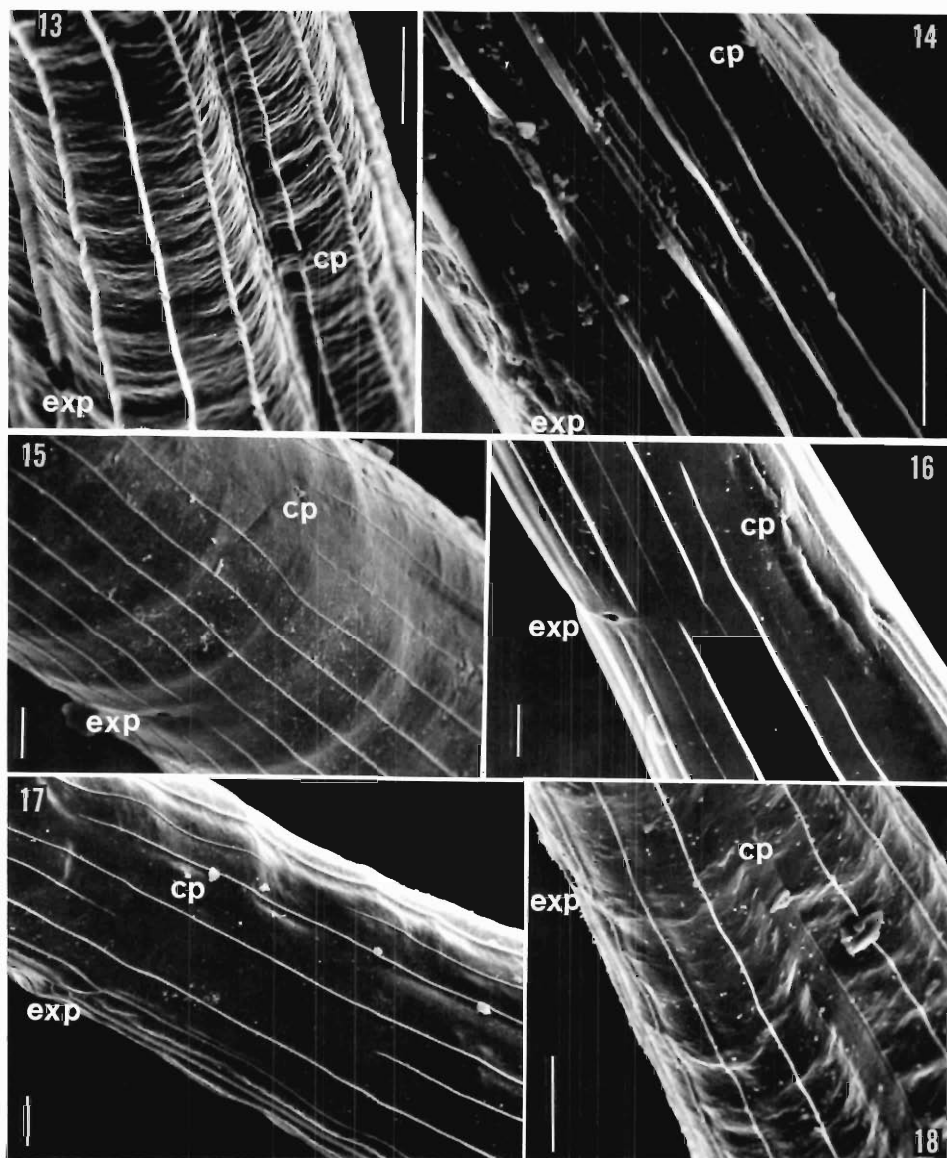
Results

Differences in the pattern of distribution and the number of cuticular ridges were found to be sufficient to identify both males and females of the six species of *Nematodirus* of domestic ruminants of North America. A new subspecies,

→

Figures 7-12. *Nematodirus* spp. of North American domesticated ruminants. Scanning electron micrographs of en face views showing perioral denticles, papillae of internal circle (ic) with sclerotized semicircular supports, amphids (a), and papillae of the external circle (ec), of which the submedians are double and the lateral papillae are single. Scale bars 5 μ m. 7. *N. filicollis*. 8. *N. davtiani*. 9. *N. helvetianus*. 10. *N. oiratianus interruptus* ssp. n. 11. *N. abnormalis*. 12. *N. spathiger*.

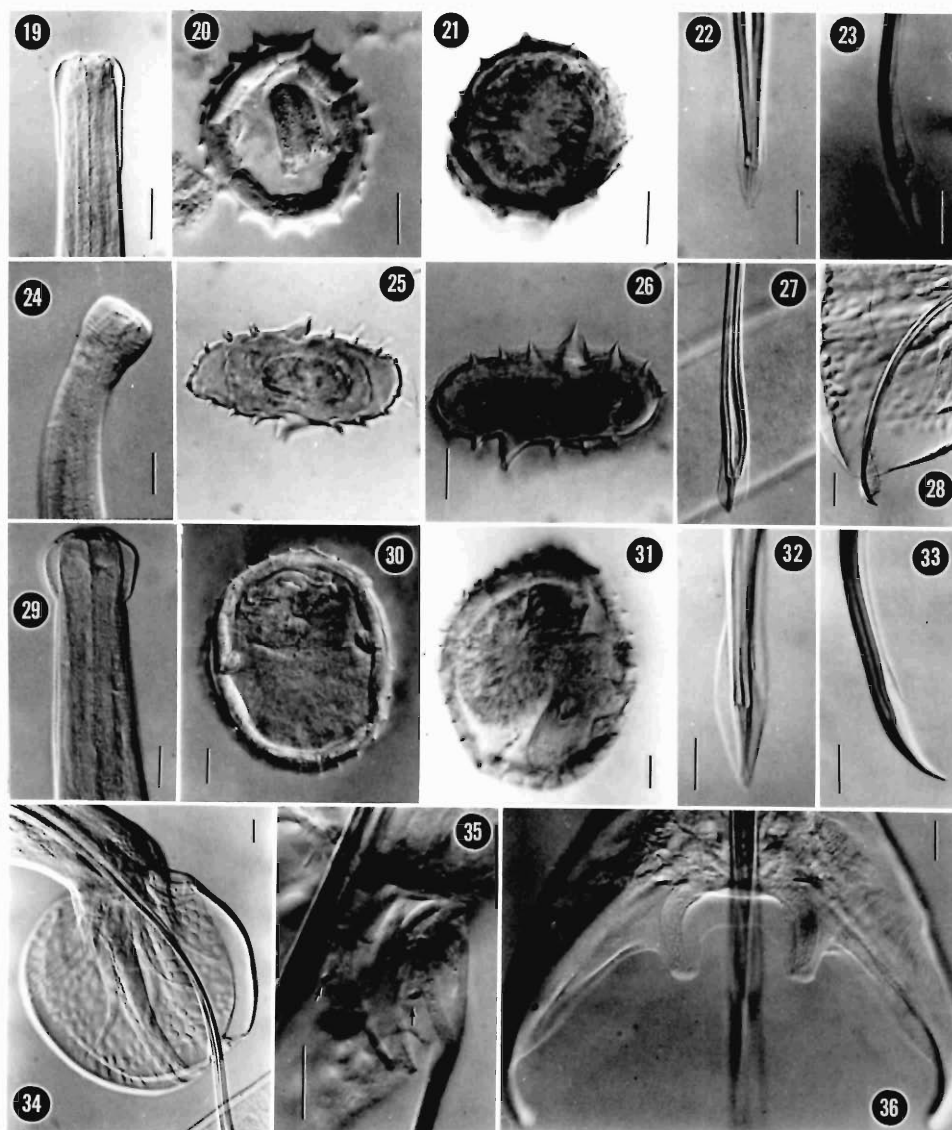




Figures 13-18. *Nematodirus* spp. of North American domesticated ruminants. Scanning electron micrographs of cervical regions showing excretory pores (exp), cervical papillae (cp), and number and morphology of cuticular ridges. Scale bars 10 μ m. 13. *N. filicollis*. 14. *N. davtiani*. 15. *N. helvetianus*. 16. *N. oiratianus interruptus* ssp. n. 17. *N. abnormalis*. 18. *N. spathiger*.

Nematodirus oiratianus interruptus ssp. n., is proposed, recognizing the distinctive cuticular ridges of the North American populations of this species.

Five of six species of *Nematodirus* of domestic ruminants of North America have variations of an 18-ridge bilaterally symmetrical system in the cervical region (from the posterior edge of the cephalic expansion to the level of the excretory pore and cervical papillae) (Figs. 1, 2, 4-6). The sixth species, *N. helvetianus*, has



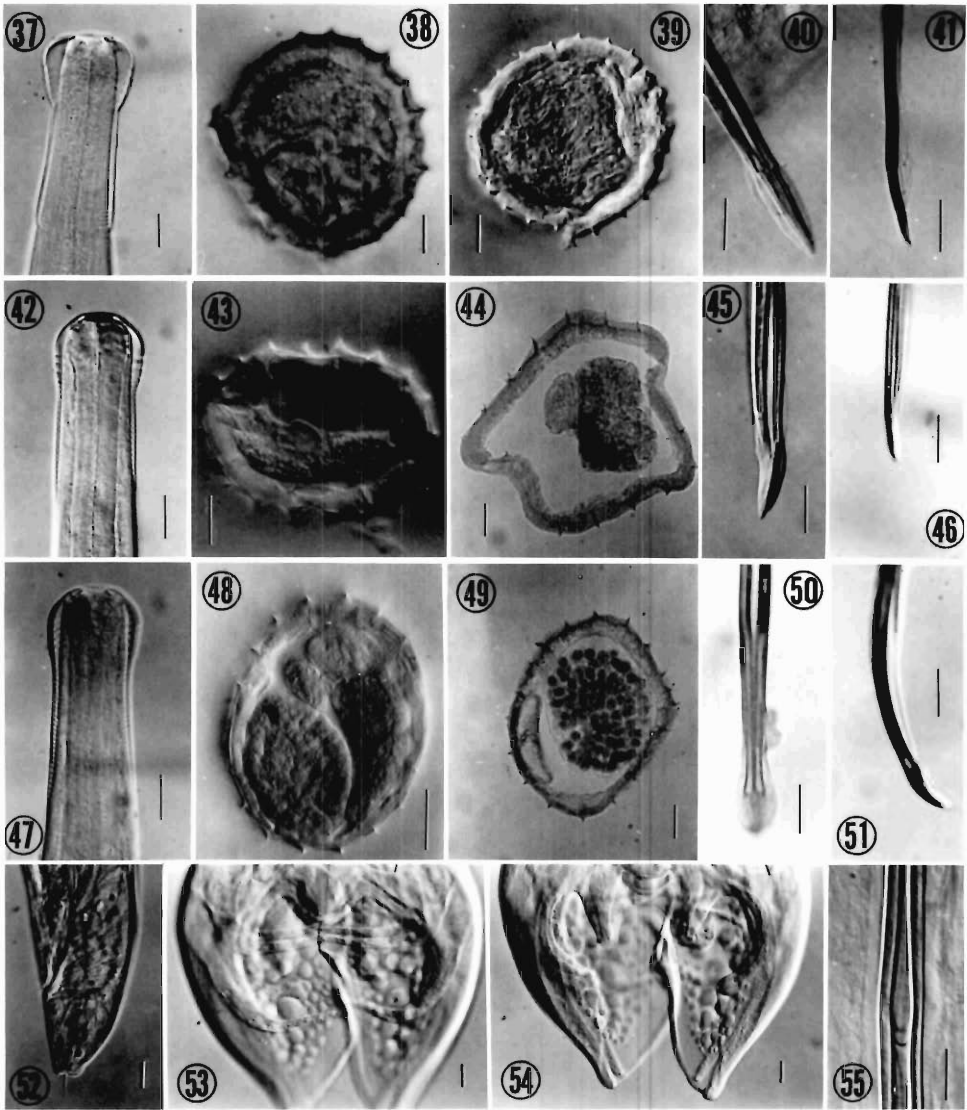
Figures 19-23. *Nematodirus filicollis*. Scale bars 20 μ m. 19. Cephalic expansion. 20. Postcervical cross section, showing 20 ridges. 21. Midbody cross section, showing 14 ridges. 22. Spicule tips, dorsoventral view. 23. Spicule tips, lateral view.

Figures 24-28. *Nematodirus davtianii*. Scale bars 20 μ m. 24. Cephalic expansion. 25. Postcervical cross section, showing 18 ridges. 26. Midbody cross section, showing 14 ridges. 27. Spicule tips, dorsoventral view. 28. Spicule tips, lateral view.

Figures 29-33. *Nematodirus helvetianus*. Scale bars 20 μ m. 29. Cephalic expansion. 30. Postcervical cross section, showing 26 ridges. 31. Midbody cross section, showing 34 ridges. 32. Spicule tips, dorsoventral view. 33. Spicule tips, lateral view.

Figures 34, 35. *Nematodirus davtianii*. Scale bars 20 μ m. 34. Copulatory bursa, lateral view. 35. Dorsal ray, arrow at spurlike ramus.

Figure 36. *Nematodirus spathiger*. Scale bar 20 μ m. Copulatory bursa, dorsal view.



Figures 37-41. *Nematodirus oiratianus interruptus* ssp. n. Scale bars 20 μ m. 37. Cephalic expansion. 38. Postcervical cross section, showing 22 ridges. 39. Midbody cross section, showing 22 ridges. 40. Spicule tips, dorsoventral view. 41. Spicule tips, lateral view.

Figures 42-46. *Nematodirus abnormalis*. Scale bars 20 μ m. 42. Cephalic expansion. 43. Postcervical cross section, showing 18 ridges. 44. Midbody cross section, showing 18 ridges. 45. Spicule tips, dorsoventral view. 46. Spicule tips, lateral view.

Figures 47-51. *Nematodirus spathiger*. Scale bars 20 μ m. 47. Cephalic expansion. 48. Postcervical cross section, showing 18 ridges. 49. Midbody cross section, showing 18 ridges. 50. Spicule tips, dorsoventral view. 51. Spicule tips, lateral view.

Figures 52-55. *Nematodirus oiratianus interruptus* ssp. n. Scale bars 20 μ m. 52. Female tail, lateral view. 53. Copulatory bursa, ventral view. 54. Copulatory bursa, dorsal view. 55. Spicules, showing junction of proximal and middle thirds where they become joined by a common membrane.

two extra pairs of ridges, for a total of 26 in the cervical region (Fig. 3). The ridges in Figures 1–6 are numbered in two directions (dorsally and ventrally), beginning at the right cervical papilla.

The six species can be divided into two groups by characters A–E (Fig. 56). Group I, with two species (*N. filicollis* and *N. davtianii*), has a cervical ridge pattern in which the lateral ridges are extended more anteriorly than in Group II. The lateralmost pairs of cervical ridges (pairs 1 and 9) extend more than one-third of the cervical distance and ridge pairs 2, 3, 7, and 8 also extend anteriorly, pairs 3 and 8 nearly, or actually, reaching the cephalic expansion (Figs. 1, 2).

The dorsal and ventral ridges of the two species are finlike (Figs. 13, 14), the cephalic expansion is usually less than twice as long as thick (Figs. 19, 24), and the number of ridges is reduced to 10–14 around midbody or slightly posterior to midbody (Figs. 21, 26). In the last quarter of the body all dorsal ridges disappear and the number of ventral ridges increases to 11 in *N. davtianii* and 15 in *N. filicollis*. In addition to the cuticular characteristics that they share, *N. davtianii* and *N. filicollis* share the characteristics of a small number of perioral denticles (Figs. 7, 8) and a large bursa with numerous bosses in which the dorsal ray is not set off in a separate lobe (Becklund and Walker, 1967) (Fig. 34).

The two species of Group I can be separated by cuticular, bursal, and spicular differences. Females of the rare species *N. davtianii* were not available, so a direct comparison of females was not possible. The only known description of female *N. davtianii* is that of *N. davtianii alpinis* Biocca, Balbo, and Costantini, 1974. *Nematodirus davtianii* differs from *N. filicollis* by having exceptionally large dorsalmost and ventralmost (pair no. 5) ridges (Figs. 25, 26) and by lacking the extra pairs of fine ridges posterior to the cervical papillae of *N. filicollis* (Figs. 1, 2).

Group II includes four of the six species (*N. helvetianus*, *N. oiratianus*, *N. abnormalis*, and *N. spathiger*; Figs. 3–6) that share the cuticular characteristics of the lateralmost cervical ridges extending less than one-third of the cervical distance, the cephalic expansions more than twice as long as thick (Figs. 29, 37, 42, 47), the dorsal and ventral ridges not as finlike as in *N. davtianii* and *N. filicollis*, and the number of ridges near midbody 18 or more (Figs. 31, 39, 44, 49). The four species of Group II also share the characteristics of numerous perioral denticles (Figs. 9–12) and small bursae in which the dorsal rays are set off in separate lobes (Figs. 36, 54).

The four species of Group II can be separated by cuticular differences. In addition, differences in the spicules and copulatory bursae were reported earlier by Becklund and Walker (1967) and Stringfellow (1968). Only cuticular differences will be described below, but other differences are included in the cladogram and in the key to species. The descriptions of the cuticle include both males and females unless noted otherwise.

Nematodirus helvetianus can be identified by its large number of ridges. Five dorsal and five ventral ridges reach the cephalic expansion (Fig. 3), four more than in the other three species of Group II, in which only three dorsal and three ventral ridges reach the cephalic expansion. Just posterior to the excretory pore of *N. helvetianus* there are 26 ridges (Figs. 3, 15, 30), and at midbody there are 34 (Fig. 31). In the last quarter of the body of the male all dorsal ridges are lost without an increase in the number of ventral ridges.

***Nematodirus oiratianus interruptus* ssp. n.**
(Figs. 4, 10, 16, 37–41, 52–55)

When *N. oiratianus* from other parts of the world (Table 1) were compared with *N. oiratianus* of North America, the North American specimens were found to differ, but only in the presence of discontinuities in the ridges of the cervical region (Table 3). Because the North American specimens of *N. oiratianus* were from a wide range of hosts from all over the western half of North America (Table 1), the discontinuous ridges are believed to represent a true genetic difference between the North American populations and other populations of *N. oiratianus*. In order to provide a handle for the information pertaining to this unique population of *N. oiratianus* that differs both morphologically and geographically, we propose a subspecies designation for it.

Both males and females of the new subspecies can be identified by the interruptions in the cuticular ridges in the cervical region (Figs. 4, 16). Posterior to the cervical region the ridges gradually become continuous. A strong pair of ridges extends to the level of the cervical papillae and another finer pair of ridges begins a bit more posteriorly (Fig. 4), to bring the total number of ridges just posterior to the cervical region to 22–26 (Fig. 38). Before reaching midbody one or both of the fine lateral ridges are lost on each side so that 22–24 ridges are present at midbody (Fig. 39). Unlike the other species, which lose dorsal ridges a millimeter or more anterior to the bursa, both *N. oiratianus interruptus* and *N. o. oiratianus* retain their dorsal ridges to about 0.500 mm anterior to the bursa.

HOSTS: *Ovis canadensis* (type); others (Table 1).

LOCATION: Small intestine.

LOCALITY: Montana, U.S.A. (type); others (Table 1), all North America.

SPECIMENS DEPOSITED: USNM Helm. Coll., USDA, BARC-East, Beltsville, Maryland 20705; holotype, male, No. 77397; allotype, female, No. 77398; paratypes, Nos. 77399–77408, 38054, 39011, 56783, 59498, 59209, 69367, 75420, 75421.

Nematodirus abnormalis can be recognized by the interruptions in the ridges of pairs 2 and 8 (Figs. 5, 17) at the level of the excretory pore and cervical papillae. There are 18 ridges for most of the postcervical body length (Figs. 43, 44), except in the posterior quarter of the male, where the dorsal ridges disappear and the ventral ridges increase in number from nine to a total of 12. An examination of *N. abnormalis* from South America and Australia found a consistent pattern in this species from different parts of the world. In the specimens from Australia an additional interruption in the ridges of pairs 2 and 8 occurred about 300 μ m posterior to the anterior interruption. The posterior interruption was found in only one specimen from North America.

An examination of female *N. abnormalis* identified by the unique cuticular characters described above revealed that the vulva of this species is located more anteriorly than in any of the other species from North American ruminants (Table 2).

Nematodirus spathiger can be recognized by its lack of most of the specialized cuticular characteristics described for the other species (Fig. 6). It is most similar to *N. abnormalis*, but it lacks the interruptions in pairs 2 and 8. *Nematodirus spathiger* is the most variable of the six species. In some specimens the ridges of

Table 2. Morphometrics of female *Nematodirus* spp. from domestic ruminants of North America.

Characters†	Species of <i>Nematodirus</i>					
	<i>N. filicollis</i>	<i>N.* daviani</i>	<i>N. helveticus</i>	<i>N. oiratianus interruptus</i> ssp. n.	<i>N. abnormalis</i>	<i>N. spathiger</i>
Number of specimens	6	10	2	10	9	9
Body length (mm)	14.3–17.9	10.1–13.6	29.5	8.6–20.5	11.1–16.0	12.8–19.0
Esophagus length	480–639	450–520	672–678	375–654	435–552	486–513
Excretory pore‡	380–585		672–855	375–600	231–510	375–531
Anterior ovejector						
Vestibule	129–291		222–270	96–195	150–243	165–270
Sphincter	60–69		77–90	48–90	60–75	60–81
Infundibulum	147–270		189–324	195–515	165–240	210–300
Posterior ovejector						
Vestibule	135–255		156–210	90–240	165–270	150–216
Sphincter	54–75		77–96	54–96	60–75	57–78
Infundibulum	177–309		285–336	159–312	186–270	210–285
Vulva position as percentage‡ of body length (%)	66–73	74–78	73–76	67–79	55–63	66–72
Egg length	146–167	200–205	186–224	189–230	172–200	172–217
Egg width	68–88	90–105	86–111	79–120	101–116	95–114
Tail length	45–69	50–80	71–94	51–75	51–135	63–84

* Measurements from Biocca et al. (1974).

† Measurements in micrometers unless indicated otherwise.

‡ From anterior end of body.

pairs 1 and 9 extend anterior to the cervical papillae, but in others they do not reach that level (Fig. 18).

Discussion

The differences in pattern and number of cuticular ridges among the six species of *Nematodirus* are sufficient to identify males and females to species. This is the

Table 3. Morphometrics of *Nematodirus oiratianus* ssp. males.*

Characters†	Subspecies	
	<i>N. oiratianus interruptus</i> ssp. n.	<i>N. oiratianus oiratianus</i>
Number of specimens	10	10
Body length (mm)	8.3–12.4	7.2–14.8
Esophagus length	375–525	396–493
Excretory pore	417–546	323–608
Spicule length	729–907	665–900
Spicule tip length	36–39	28–40
Length of fusion proximal to spicule tips	6–23	13–29
Percentage of spicules joined by common membrane (%)	64–66	61–67
Bursa length	90–141	80–150
Dorsal ray length	36–54	38–60

* Morphometrics of females of *N. oiratianus interruptus* ssp. n. in Table 2; females of *N. oiratianus oiratianus* unavailable.

† Measurements in micrometers unless indicated otherwise.

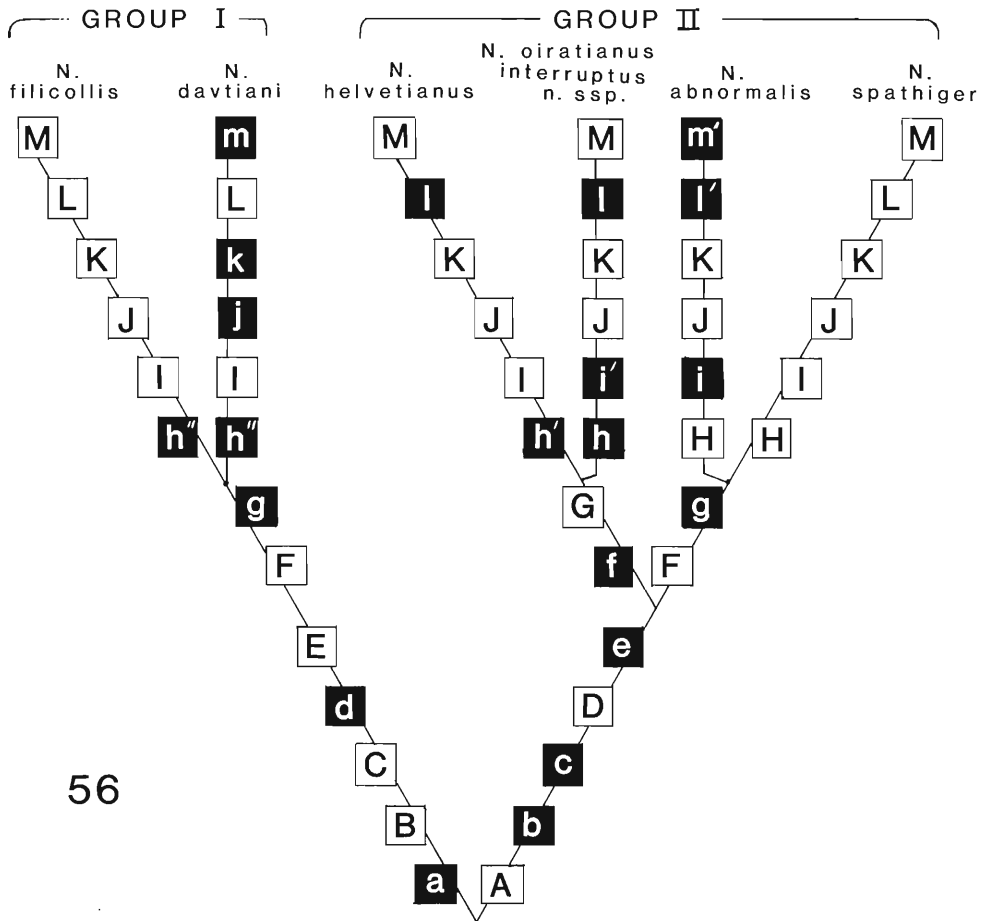


Figure 56. Cladogram of possible evolutionary relationships of *Nematodirus* spp. of domestic ruminants of North America. Capital letters in open boxes indicate generalized or ancestral character states; lowercase letters in black boxes indicate specialized character states: (A) lateralmost cervical ridges extend less than $\frac{1}{3}$ cervical distance; (a) lateralmost cervical ridges extend more than $\frac{1}{3}$ cervical distance; (B) 30-35 perioral denticles, (b) 50-65 perioral denticles; (C) dorsal rays not in separate lobes from laterals, (c) dorsal rays in separate lobes from laterals; (D) ridges not finlike, (d) ridges finlike; (E) cephalic expansion more than twice as long as broad, (e) cephalic expansion less than twice as long as broad; (F) 18 postcervical ridges, (f) 22-26 postcervical ridges; (G) no added ridges in posterior quarter of male, (g) ridges added ventrally in posterior quarter of male; (H) 18 midbody ridges, (h) 22-24 midbody ridges, (h') 34 midbody ridges, (h'') 14 midbody ridges; (I) ridges continuous, (i) ridge pairs 2 and 8 discontinuous at cervical papillae, (i') all cervical ridges discontinuous; (J) two short rami at end of dorsal ray, (j) one spurlike ramus at middle of dorsal ray; (K) dorsalmost and ventralmost ridges not hypertrophied, (k) dorsalmost and ventralmost ridges more than twice height of other ridges; (L) spicule tips short (18-26 μm) and symmetrical, (l) spicule tips elongate (36-38 μm), (l') spicule tips asymmetrical; (M) vulva near junction of middle and posterior fourths of body, (m) vulva in posterior fourth of body, (m') vulva in third fourth of body.

first character found to be useful for identifying female *Nematodirus* to species. Because this character can be observed in living and fresh-frozen specimens, as well as in fixed and cleared specimens, many kinds of studies requiring identified females and males are now possible.

The study of females identified by cuticular characters has so far revealed few additional differences among the species (Table 2). Only differences in vulva position were observed. For the *Nematodirella*, which are believed to have evolved from *Nematodirus* (Durette-Desset, 1979; Lichtenfels and Pilitt, 1983), a more anterior vulva and longer spicules were considered to be evolved characteristics. We interpret the results in Table 2 to indicate that the ancestral (most common) vulva position was near the junction of the middle and posterior thirds. The more anterior vulva of *N. abnormalis* is believed to be a specialized character state. The more posterior vulva reported by Biocca et al. (1974) for *N. davtiani alpinus* may also represent a specialized character state that has evolved in the opposite direction. Of the six species, *N. abnormalis* has the most anterior vulva (Table 2). This character was consistent when samples of nematodes from other widely separated parts of the world were studied (Table 1).

Although the six species that were studied represent less than half of the species of *Nematodirus* parasitic in feral and domestic ruminants of the world, some relationships among the six species are apparent. Thirteen morphological characters and 31 character states were used to reconstruct possible relationships of the six species (Fig. 56), and to construct the following key to species.

Key to *Nematodirus* of Domestic Ruminants of North America

1. Lateralmost pair of cuticular ridges extends anteriorly more than one-third of distance between excretory pore and cephalic expansion (Figs. 1, 2). Cephalic expansion about twice as long as broad, or shorter (Figs. 19, 24). Number of cuticular ridges in region posterior to midbody (the third quarter) 10–14 (Figs. 21, 26). Copulatory bursa large, without separate dorsal lobes (Fig. 34). Spicule tips short (18–30 μ m) and symmetrical (Figs. 22, 23, 27, 28)..... 2
- Lateralmost pair of cuticular ridges extends less than one-third of distance between excretory pore and cephalic expansion (Figs. 3–6). Cephalic expansion more than twice as long as broad (Figs. 29, 37, 42, 47). Number of cuticular ridges in midbody or in third quarter 18–34 (Figs. 31, 39, 44, 49). Copulatory bursa small, with separate dorsal lobes (Fig. 36). Spicule tips elongate (Figs. 32, 33, 40, 41), asymmetric (Figs. 45, 46), or short and broad (Figs. 50, 51)..... 3
2. Extra pairs of ridges present bilaterally in postcervical region for total of 22 ridges (Figs. 1, 13). Dorsalmost and ventralmost ridges not more than twice height of other ridges (Figs. 20, 21). Dorsal rays end with two short rami (similar to Fig. 36). Spicule fused anterior to tips; tips with narrow membrane (Figs. 22, 23) *N. filicollis*
- Extra pair of ridges absent, total of 18 ridges in postcervical region (Figs. 2, 14, 25). Dorsalmost and ventralmost ridges more than twice height of other ridges (Figs. 25, 26). Dorsal rays with short spurlike ramus at middle of ray and single point at distal end (Fig. 35). Spicule fused only at tips; tips with broad membrane (Figs. 27, 28) *N. davtiani*
3. Twenty-two to 26 ridges just posterior to excretory pore (Figs. 3, 4, 15, 16, 30, 38). Twenty-two to 34 ridges near midbody (Figs. 31, 39). Number of ventral ridges does not increase in posterior quarter of male..... 4

- Eighteen ridges just posterior to excretory pore (Figs. 5, 6, 17, 18, 43, 48).
 Eighteen ridges near midbody (Figs. 44, 49). Number of ventral ridges increases in posterior quarter of male 5
4. Ridges continuous in cervical region (Figs. 3, 15). Number of ridges in postcervical region 26 (Figs. 3, 15, 30); in midbody 34–36 (Fig. 31). Only ventral ridges extend within 500 μ m of bursa. Spicule tips with ventral membrane proximal to tip (Fig. 33) *N. helvetianus*
 Ridges discontinuous in cervical region (Figs. 4, 16). Number of ridges in postcervical region 22–26 (Figs. 4, 16, 38); in midbody 22–24 (Fig. 39). Both dorsal and ventral ridges extend to within 500 μ m of bursa. Spicule tips with ventral membrane at middle of tip (Fig. 41)
 *N. oiratianus interruptus* ssp. n.
5. Ridges of pairs 2 and 8 discontinuous at level of cervical papillae (Figs. 5, 17). Vulva 55–63% of body length from anterior end (Table 2). Spicule tips asymmetric and twisted (Figs. 45, 46) *N. abnormalis*
 Ridges in cervical region continuous (Figs. 6, 18). Vulva 66–72% of body length from anterior end (Table 2). Spicule tips symmetrical; short with broad membrane, cup-shaped in lateral view (Figs. 50, 51) *N. spathiger*

Acknowledgments

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***Diplotrriaena utae* sp. n. (Nematoda: Diplotrriaenoidea) in the Gray Jay (*Perisoreus canadensis* (L.)) in Ontario, Canada**

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ABSTRACT: The presence of numerous rounded elevations on the apex of the trident distinguishes *D. utae* sp. n. from all other species in the genus. *Diplotrriaena lagopusi* Olsen and Braun, 1971 is regarded as a synonym of *D. couturieri* Dollfus, 1956.

Nematodes belonging to the genus *Diplotrriaena* Henry and Ozoux, 1909 were recovered from the abdominal air sacs of gray jays (*Perisoreus canadensis* (L.)) in Onakawana, Ontario, Canada. These helminths represent a new species named in honor of Mrs. Uta Renate Strelive of the Department of Zoology, University of Guelph, Ontario.

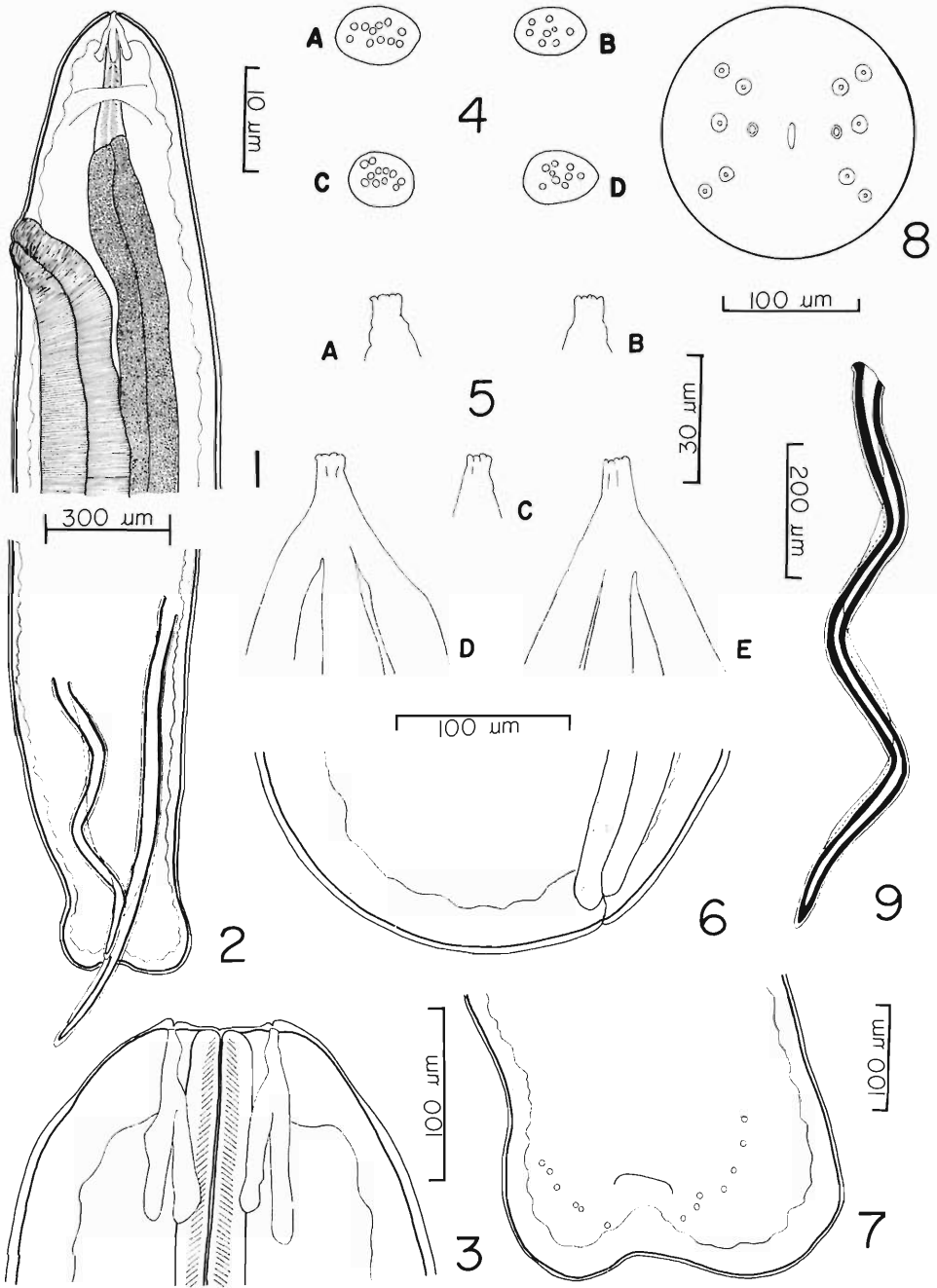
Types of *D. lagopusi* Olsen and Braun, 1971 from *Lagopus leucurus altipetens* Osgood in Colorado were borrowed from the United States National Museum Helminthological Collection (No. 70518) through the courtesy of Dr. Ralph Lichtenfels, United States Department of Agriculture, Beltsville, Maryland. They were compared with the specimens from gray jays and with *D. couturieri* as redescribed by Anderson (1959).

***Diplotrriaena utae* sp. n. (Figs. 1–9)**

DESCRIPTION: Cuticle of body with faint transverse striations. Four pairs of submedian cephalic papillae present. Amphids and pores of tridents prominent. Tridents medium sized, arms well separated with bases often difficult to discern. Apex of trident flattened, with varying numbers of rounded elevations observable in lateral and apical views.

MALE ($N = 2$): Length 16.0, 18.5 mm. Maximum width 500, 550 μm . Trident length 100, 110 μm . Nerve ring 170, 200 μm from anterior extremity. Total esophageal length 3.2, 3.6 mm; muscular portion 0.3 mm and glandular portion 2.9, 3.3 mm. Right spicule 680, 900 μm and left spicule 1,050, 1,250 μm in length. Right spicule alate with three twists. Left spicule slender with slight ventral curve. Anus 60, 105 μm from caudal extremity. Caudal extremity with lateral expansions. Caudal papillae inconspicuous and arranged in single row on either side of anus.

FEMALE ($N = 9$): Length 42.4 (34.5–56.0) mm. Maximum width 620 (580–700) μm . Nerve ring 193 (160–210) μm from anterior extremity. Trident length 125 (115–140) μm . Total esophageal length 4.3 (3.8–4.8) mm; muscular portion 0.3 (0.2–0.3) mm and glandular portion 4.0 (3.6–4.4) mm. Vulva with prominent protuberance, 531 (400–620) μm from anterior extremity. Vagina surrounded by thick muscular fibers, 1.6 (1.1–1.9) mm in length, directed posteriorly into two uteri packed with thick-shelled eggs measuring 37 (34–38) μm by 54 (50–57) μm . Caudal extremity rounded. Anus subterminal.



Figures 1-9. *Diplotriaena utae* sp. n. 1. Anterior region of female, lateral view. 2. Posterior region of male, ventral view. 3. Head region of female, dorsoventral view. 4A, B, C, D. Tips of tridents, apical view. 5A, B, C, D, E. Trident, showing variation in apex, lateral view. 6. Tail of female, lateral view. 7. Tail of male, ventral view. 8. Anterior end female, en face view. 9. Right spicule, ventral view.

HOST: *Perisoreus canadensis* (L.) (Corvidae).

LOCALITY: Onakawana, Ontario, Canada.

LOCATION: Abdominal air sacs.

SPECIMENS: National Museums of Canada, Ottawa, Ontario K1A 0M8; holotype (male), NMCIC(P) 1982-0121; allotype (female), NMCIC(P) 1982-0122; paratypes, NMCIC(P) 1982-0123. USNM Helminthological Collection, USDA, Beltsville, Maryland 20705: paratypes, No. 77068.

Remarks

Diplotriaena utae is readily distinguished from other described members of the genus by the flattened apex of the trident with its numerous rounded elevations. *Diplotriaena utae* resembles *D. couturieri* Dollfus, 1956 from *Lagopus mutus helveticus* (Thienemann) (Tetraonidae), but the apex of the trident of the latter species has two grooves and lacks elevations; comparison of *D. lagopusi* with *D. couturieri* (see Anderson, 1959) indicates that the former is a synonym of the latter.

Three species of *Diplotriaena* have previously been reported from Corvidae in North America. *Diplotriaena tricuspis* (Fedtschenko, 1874) has been reported in the blue jay (*Cyanocitta c. cristata* L.) and the common crow (*Corvus b. brachyrhynchos* Brehm) (Anderson, 1959; Cawthorn and Anderson, 1980). *Diplotriaena hepatica* (Walton, 1927) and *D. multituberculata* Walton, 1927 have been found in the scrub jay (*Aphelocoma c. coerulescens* (Bosc) = *Aphelocoma floridana*, *A. cyanea floridana*). The status of the latter two species of *Diplotriaena* is uncertain, and Anderson (1959) suggested they may be synonyms of *D. tricuspis*.

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***Heligmosomoides thomomyos* sp. n. (Nematoda: Heligmosomidae)
from Pocket Gophers, *Thomomys* spp. (Rodentia: Geomyidae),
in Oregon and California**

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ABSTRACT: The nematode *Heligmosomoides thomomyos* sp. n. is described from the small intestine of *Thomomys bulbivorus* and *T. bottae*. This is the first report of *Heligmosomoides* from rodents of the family Geomyidae.

Nematodes of the genus *Heligmosomoides* Hall, 1916 occur most commonly in arvicolid rodents. Of the 26 known species of the genus, 11 have been recorded from Nearctic rodents (Rausch and Rausch, 1973). In North America, *Heligmosomoides* spp. have been recorded not only from voles, but also from cricetids and murids. *Heligmosomoides* spp. characteristically inhabit the small intestine or cecum of their host, where they usually are found tightly coiled around the intestinal or cecal villi (Durette-Desset, 1971).

Nematodes representing an undescribed species of *Heligmosomoides* were found by us in the small intestine of pocket gophers (Geomyidae) of two species, *Thomomys bulbivorus* (Richardson), from Benton County, Oregon, and *T. bottae* (Eyndoux and Gervais), from Humboldt County, California (collected respectively by S.L.G. and D.P.J.). Rodents of the genus *Thomomys* have an extensive geographic range in central and western North America. *Thomomys bulbivorus* is endemic to the Willamette Valley of Oregon, and *T. bottae* occurs from southwestern Oregon southward to Arizona and northern Mexico (Hall and Kelson, 1959; Ingles, 1965).

It is the purpose of the present paper to describe this nematode, which is the first species of *Heligmosomoides* to be recorded from rodents of the family Geomyidae.

Materials and Methods

Gophers were necropsied as soon as possible after collection. Organs of the gastrointestinal tract of *T. bulbivorus* were opened and washed separately in fresh water. The intestinal contents were examined in a Petri dish marked with a grid, under a dissecting microscope. The intestines of *T. bottae* were opened and examined under a dissecting microscope without washing. All nematodes were removed, preserved in 70% ethanol, cleared by evaporation of 70% ethanol with 5%; glycerine, and mounted whole in glycerine-jelly or in glycerine and 2% lactic acid. The structure of the synlophe (Durette-Desset, 1971) was determined from transverse sections taken approximately 2 mm from the anterior and posterior ends, and near midbody of both male and female specimens. The ovejector was dissected from one female for detailed study.

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The type specimen, No. 205 H, of *Heligmosomoides montanus* Durette-Desset, 1968 was obtained from the Muséum National d'Histoire Naturelle, Paris, for comparative studies. Two cotype specimens, one male (No. 2288) and one female (No. 30455), of *Heligmosomoides longispiculatus* (Dikmans, 1940) were obtained from the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 for comparisons with our material.

Results

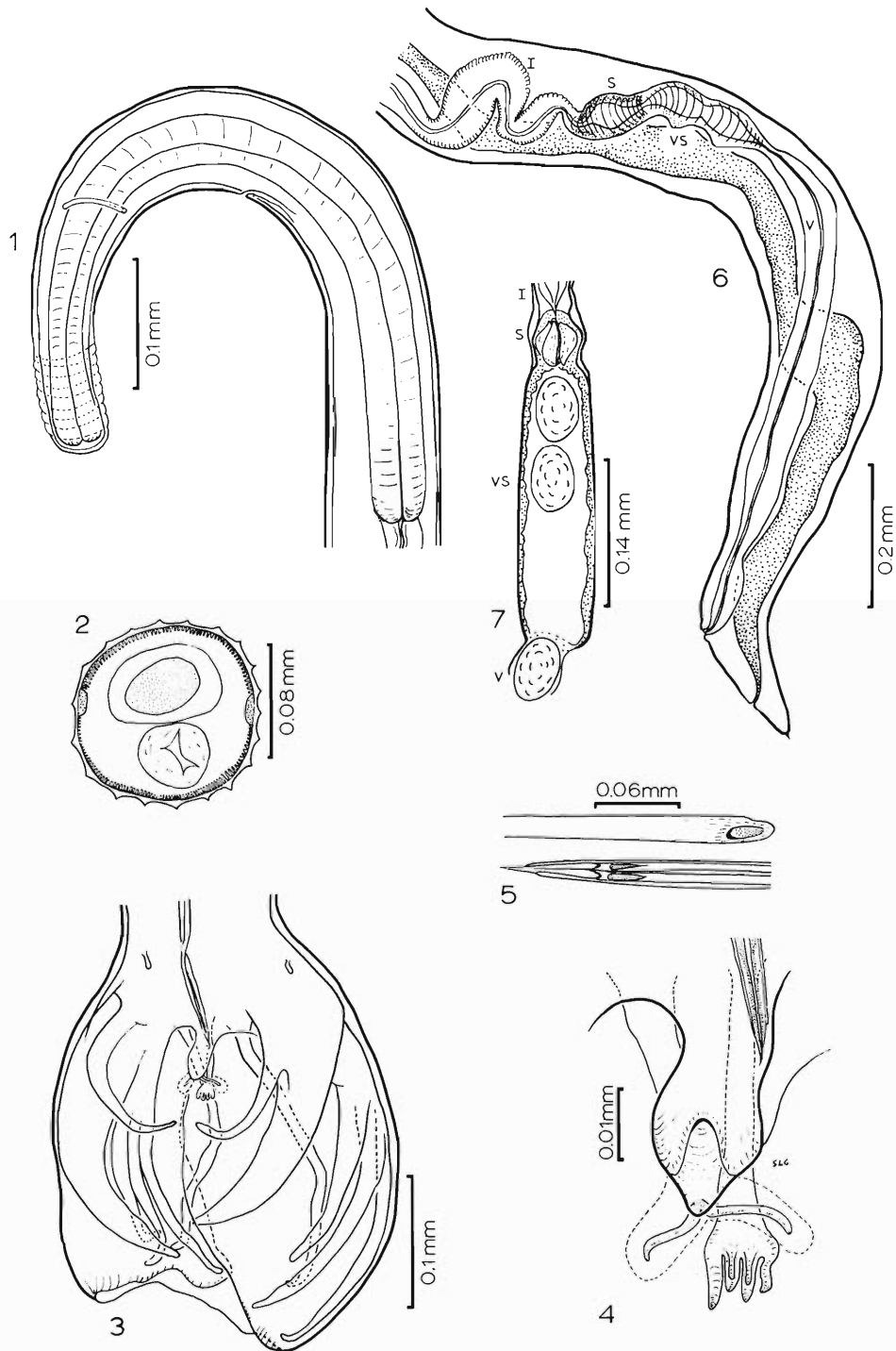
The nematodes considered in this study were usually found tightly coiled and free in the intestinal contents. The following description is based on five male and 15 female specimens. Measurements are in micrometers unless otherwise indicated; means are in parentheses.

Heligmosomoides thomomyos sp. n.

(Figs. 1-7)

DESCRIPTION: MALE: Length 5.12-6.45 mm (5.60 mm). Maximum diameter, at level about $\frac{7}{8}$ of length from anterior extremity, 85-124 (112). Width at anterior end 26-40 (35). Width at base of esophagus 89-112 (102); at level just anterior to bursa 76-120 (101). Cephalic inflation about 37 by 62. Excretory pore 225-320 (294) and nerve ring 180-263 (205) from anterior extremity. Distance from nerve ring to excretory pore 67-110 (89) (Fig. 1). Cuticle about 3 thick, provided with 18 or 19 longitudinal ridges, 3-7 high and decreasing in height dorsad. Esophagus 429-666 (577) long and 89-112 (102) wide at base. Spicules subequal, 3.40-3.73 mm (3.60 mm) long, with attenuated slender tips, joined at distal end (Fig. 5); each consisting of 2 cylindrical processes discernible in cross section. Bursa somewhat asymmetrical, with the sinistral lobe slightly larger. One pair of prebursal papillae, 11-28 (26) long, present ventrally (Fig. 3). Genital cone well developed, possessing 2 slender projections about 19 long by about 1 wide (Fig. 4). Dorsal ray complex, 54-62 (58) long, having 6 slender projections, of which the medial pair is about 12 long; 1 lateral pair about 10 long; 1 dorsal pair 6 long, lying just dorsal to and overlapping medial pair. Slender externodorsal rays 120-182 (148), mediolateral rays 113-220 (166), and posterolateral rays 115-220 (184) in length. Anteroventral rays 80-113 (99) long; thick posteroventral rays 120-265 (207) long. Bursal membrane well developed.

FEMALE: Length 8.70-11.95 mm (10.21 mm). Maximum diameter at level about $\frac{2}{5}$ of length from anterior extremity 131-249 (191). Width at anterior end 21-57 (37). Width at level of nerve ring 54-86 (70); at base of esophagus 83-131 (107); at level of vulva 63-125 (82); at level of anus 21-55 (39). Cephalic inflation 34-69 wide by 47-100 long. Excretory pore 151-389 (295) and nerve ring 85-250 (176) from anterior extremity. Distance from nerve ring to excretory pore 52-140 (104). Cuticle provided with 18-20 longitudinal ridges, 2-11 high, decreasing in height dorsad (Fig. 2). Esophagus 539-729 (628) long and 33-63 (45) wide at base. Anus 21-55 (39) anterior to end of tail; tail provided with a caudal spine 7-13 long (Fig. 6). Vulva 106-253 (158) anterior to end of tail (to base of caudal spine). Vagina 605-1.0 mm (841) long. Ovejector 103-324 (227) long, with anterior chamber (sphincter) averaging 46 by 58, posterior chamber (vestibule) averaging 88 by 150, and infundibulum averaging 39 by 84 (Figs. 6, 7). Thin-shelled eggs 38-47 by 61-68 (44 by 64) (Fig. 7).



Figures 1-7. *Heligmosomoides thomomyos* sp. n. 1. Male, cephalic end, lateral view. 2. Female, synlophe. Transverse section through vagina approximately 0.5 mm anterior to vulva. 3. Male, bursa, ventral view. 4. Male, details of dorsal ray and genital cone, ventral view. 5. Male, details of spicule.

Table 1. Morphological characteristics of *Heligmosomoides montanus*, *H. longispiculatus*, and *H. thomomys*. (Measurements in micrometers unless otherwise indicated; asterisks indicate measurements obtained from type material in 1982.)

Character	<i>H. montanus</i> Durette-Desset, 1968	<i>H. longispiculatus</i> (Dikmans, 1940)	<i>H. montanus</i> sp. n.
Total length (mm): Male	6.0	5.5–6.5	5.12–6.45
Female	11.4	12–13	8.70–11.95
Maximum width: Male	95	140	85–124
Female	150	150–160	131–249
Cephalic inflation			
(length × width): Male	—	—	37 × 62
Female	—	—	34–69 × 47–100
Sexes combined	40 × 60	35–40 × 70	—
Number of cuticular ridges: Male	17	?	18–19
Female	19	?	18–20
Nerve ring to anterior end: Male	200	123*	180–263
Female	180	?	85–250
Excretory pore to anterior end: Male	325–330	137*	255–320
Female	270–280	149*	151–389
Vulva to end of tail	150	210–220	106–253
Anus to end of tail: Female	50	60	21–55
Esophagus length: Male	630	—	429–666
Female	800	—	539–729
Sexes combined	—	700–800	—
Anterior chamber, ovejector	70 long	54* long	22–61 × 33–73
Posterior chamber, ovejector	110 × 390	94* long	22–97 × 59–278
Dorsal ray, length	38	?	54–61
Dorsal ray, number of terminal projections	4	?	6
Spicule length (mm)	3.9	3.9–4.0	3.42–3.73
Eggs	50 × 80	40–45 × 80–90	38–47 × 61–68

TYPE HOST: *Thomomys bottae* (Eydoux and Gervais).
SITE OF INFECTION: Anterior portion of small intestine (duodenum).
TYPE LOCALITY: Humboldt County, California (lat. 41°57'N, long. 124°05'W).
HOLOTYPE: Male, from *Thomomys bottae* (Eydoux and Gervais), U.S.N.M. Helm. Coll. No. 76610.
ALLOTYPE: Female, from *Thomomys bulbivorus* (Richardson), U.S.N.M. Helm. Coll. No. 76611.
PARATYPES: Two females and 1 male from *T. bottae*, collected by D.P.J. in July 1979 in Humboldt County, California (lat. 41°57'30"N, long. 124°05'W), U.S.N.M. Helm. Coll. No. 76612; 2 females and 1 male from *T. bulbivorus*, collected by S.L.G. on 9 June 1979 in Benton County, Oregon (lat. 44°30'N, long. 123°15'W), U.S.N.M. Helm. Coll. No. 76613.

6. Female, caudal end, lateral view. 7. Female, details of ovejector. Abbreviations: i = infundibulum; s = sphincter; vs = vestibule; v = vagina.

Comparisons

The family Geomyidae is of Nearctic origin, with a fossil record extending at least from the late Miocene (Kurtén and Anderson, 1980). The dispersal of members of some genera into northern South America occurred during the Pleistocene (Thenius, 1972). There is no evidence of the presence of the family Geomyidae in the Palearctic (Simpson, 1945), and *H. thomomyos* sp. n. therefore is compared only with Nearctic species of *Heligmosomoides*.

Heligmosomoides thomomyos sp. n. can be immediately separated from nine of 11 Nearctic congeners on the basis of spicule length. Of these, eight occur in rodents of the family Arvicolidae: *H. bullosus* Durette-Desset, 1967; *H. carolinensis* (Dikmans, 1940); *H. hudsoni* (Cameron, 1937); *H. johnsoni* Rausch and Rausch, 1973; *H. microti* Kuns and Rausch, 1950; *H. tenorai* Durette-Desset, 1967; *H. wisconsinensis* Durette-Desset, 1967; and *H. polygyrus americanus* Durette-Desset, Kinsella, and Forrester, 1972. *Heligmosomoides douglasi* Durette-Desset, Kinsella, and Forrester, 1972 is found in murids and arvicolids (Rausch and Rausch, 1973).

The remaining two Nearctic congeners, *H. montanus* Durette-Desset, 1968 and *H. longispiculatus* (Dikmans, 1940), have been recorded from arvicolid rodents: *H. montanus* in *Microtus longicaudus* (Merriam) from southeastern Alaska (Durette-Desset, 1968) and *Microtus californicus* (Peale) from northern California (Durette-Desset et al., 1972), and *H. longispiculatus* in *Ondatra zibethicus* (Linnaeus) and *Microtus pennsylvanicus* (Ord) in the northeastern United States (Washington, D.C., and New Jersey) (Dikmans, 1940).

Heligmosomoides thomomyos resembles both *H. montanus* and *H. longispiculatus* with regard to spicule length and relative dimension of the body (Table 1). However, it differs from *H. montanus* in the following details: the eggs are smaller than those of *H. montanus* (50×80), the esophagus is longer (800) in *H. montanus* (female), the posterior chamber of the ovejector of *H. montanus* is much longer (390), and the distance from the excretory pore to the anterior end in males is consistently greater in *H. montanus* (325–350).

Detailed study of the type specimen (male) of *H. montanus* revealed that the dorsal ray is as described by Durette-Desset (1968, Fig. 5). She stated (p. 195) that “La côte dorsale, longue de 38μ , se termine par 4 extrémités élargies.” This differs significantly from the dorsal ray of *H. thomomyos*, which has six fingerlike projections posteriorly.

As described by Durette-Desset (1968) and confirmed by our measurements, there is little, if any, bursal asymmetry in *H. montanus*. The bursal asymmetry of *H. thomomyos* is due to the larger left lateral rays (Fig. 3). *Heligmosomoides thomomyos* also differs in the number of cuticular ridges (crêtes) at midbody; males and females were found to have 18–19 and 18–20 longitudinal ridges, respectively, compared with 17 in the male and 19 in the female of *H. montanus* (Durette-Desset, 1968).

Heligmosomoides thomomyos differs from *H. longispiculatus* in that the latter has a relatively longer esophagus (700–800), and in the females, larger eggs ($40\text{--}45 \times 80\text{--}90$), greater total length (12–13 mm), and a greater distance from anus to tip of tail (60). Detailed examination of *H. longispiculatus* by us revealed that the asymmetrical condition of the bursa results primarily from the greatly enlarged

right ventral rays, which is in good agreement with Dikmans (1940, Fig. 1). This differs significantly from *H. thomomyos*, which has larger left lateral rays. The position of the male *H. longispiculatus* as mounted permanently on the slide precluded a determination of both the prebursal papillae and the dorsal ray. Additional material is needed before definite statements concerning these structures can be made.

Discussion

The presence of nematodes of the genus *Heligmosomoides* in North America is probably attributable to their dispersal in rodents from the Palearctic that were involved in faunal exchanges across Beringia during the Pleistocene epoch (Durette-Desset et al., 1972; Rausch and Rausch, 1973).

All rodents of the family Geomyidae lead a fossorial existence (Hall and Kelson, 1959; Russell, 1968). However, some arvicolid rodents occupy much the same habitat as geomyids. In Oregon, for example, voles such as *Microtus canicaudus* Miller use abandoned tunnels of gophers for food storage, reproduction, and shelter (Maser and Storm, 1970). Because rodents of the two families are often sympatric, and may share the same habitat, transfer of the precursor of *H. thomomyos* to geomyids might have occurred at least as early as Pleistocene time.

Thomomys mazama (Merriam), a pocket gopher that has an extensive range in western Oregon and northern California, is both parapatric with *T. bulbivorus* at the periphery of the Willamette Valley, and parapatric with *T. bottae* in southwestern Oregon (Hall and Kelson, 1959; Ingles, 1965; Maser et al., 1980). Because *H. thomomyos* is not host specific at the level of the species, it might be expected that it may also occur in *T. mazama* and perhaps in pocket gophers of other species in the Pacific Northwest.

Acknowledgments

The technical assistance of Dr. Robert L. Rausch, Virginia R. Rausch, Dr. Gerald D. Schmidt, and Matt S. Rand is gratefully acknowledged. Special thanks are expressed to Dr. Eric S. Loker and Dr. Robert M. Storm for assistance with the manuscript.

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**Koninklijke Academie voor Geneeskunde van België
Académie Royale de Médecine de Belgique**

**The “Denis Thienpont” Prize
Parasitology and Mycology**

Period 1983-1984

End of the first period: December 31, 1984

Summary of the Regulations

At the initiative of the “Janssen Research Foundation” in Beerse, Belgium, the biennial scientific prize “Denis Thienpont,” amounting to U.S.\$25,000, will be awarded under the auspices of the “Koninklijke Academie voor Geneeskunde van België” and the “Académie Royale de Médecine de Belgique.” The first presentation will take place in 1985.

The “Denis Thienpont” prize will be awarded to one investigator, possibly with at most two of his collaborators, presenting an original work—published or not—concerning fundamental or clinical research in parasitology or mycology.

Applications must be made on the appropriate form and sent to the permanent Secretary of one of the abovementioned Academies before December 31, 1984. Six copies of the submitted work(s) should be enclosed.

For more details please apply to the Secretary’s office of the “Koninklijke Academie voor Geneeskunde van België,” Hertogsstraat 1, 1000 Brussels, or the “Académie Royale de Médecine de Belgique,” 1, rue Ducale, 1000 Brussels.

***Echinocephalus overstreeti* sp. n. (Nematoda: Gnathostomatidae)
in the Stingray, *Taeniura melanopilos* Bleeker, from the
Marquesas Islands, with Comments on *E. sinensis* Ko, 1975**

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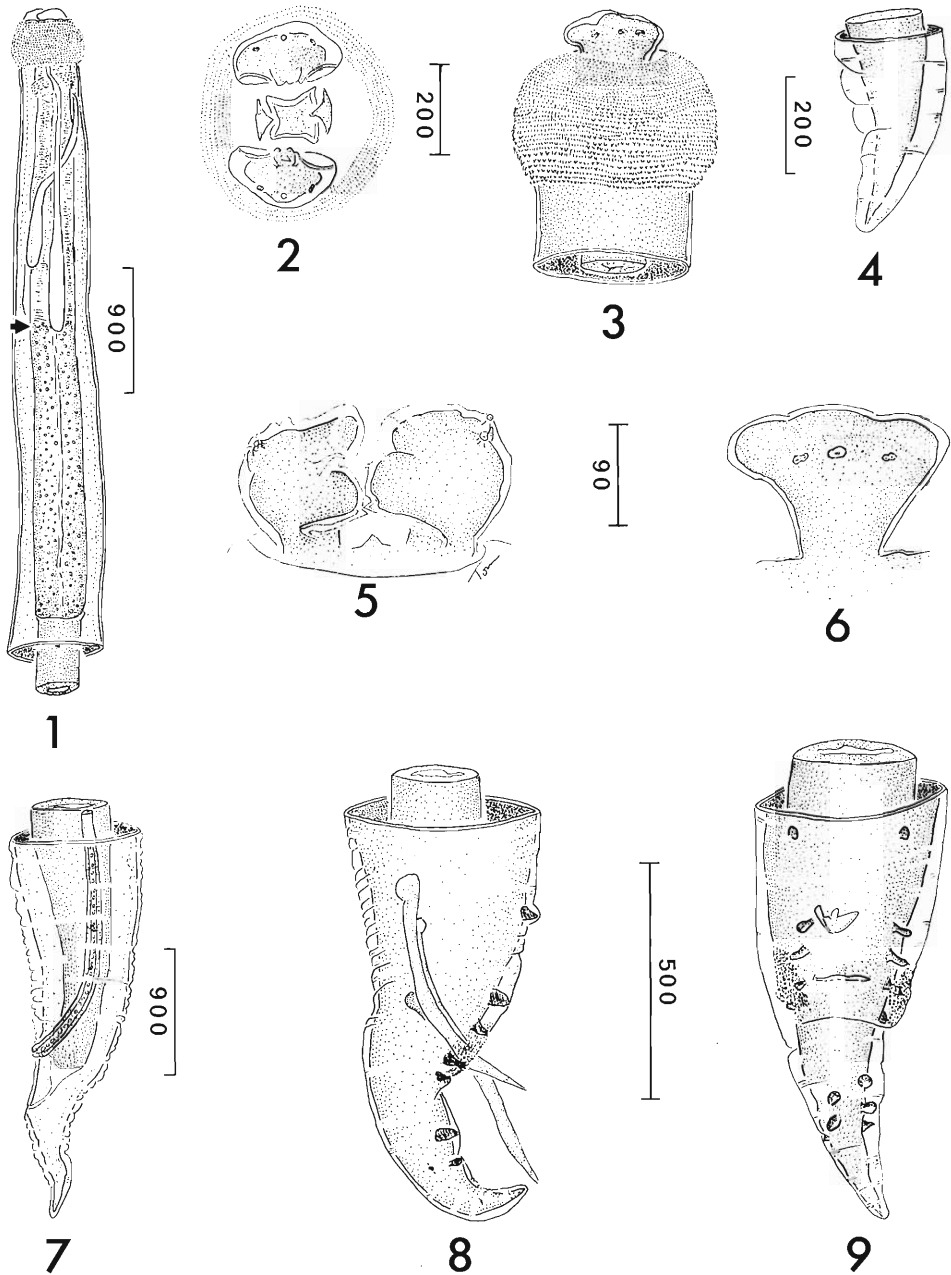
ABSTRACT: *Echinocephalus overstreeti* sp. n. infects *Taeniura melanopilos* Bleeker from the Marquesas Islands. It differs from all known species of *Echinocephalus* by possessing three preanal, one adanal, and three postanal pairs of papillae. *Echinocephalus overstreeti* is most similar to *E. sinensis* by virtue of possessing a rugose area near the cloaca of the male. The holotype of *E. sinensis* was found to possess three, rather than two, pairs of preanal papillae and modified annules on the ventral surface of posterior extremity. SEM photomicrographs of both species are included.

While examining the alimentary canal of elasmobranchs deposited at the Bernice P. Bishop Museum, Honolulu, a new species of *Echinocephalus* Molin, 1858 was discovered in the spiral valve of a stingray, *Taeniura melanopilos* Bleeker. The stingray, which had a disc length of 598 mm, yielded 49 nematodes. A brief discussion of the taxonomy of the host is reported by Randall (1980:211–212). The nematodes had been fixed in situ with formalin and stored in ethyl alcohol. The worms, removed from the host, were placed in a solution of five parts glycerin and 95 parts 70% ethyl alcohol and examined in glycerin after evaporation of the alcohol. Measurements denoting the position of the nerve ring are taken from the anterior extremity of the worm to the center of the nerve ring. For spicule ratios, the length of the left spicule equals one. All measurements are in micrometers unless otherwise stated, and figures were drawn with the aid of a drawing tube.

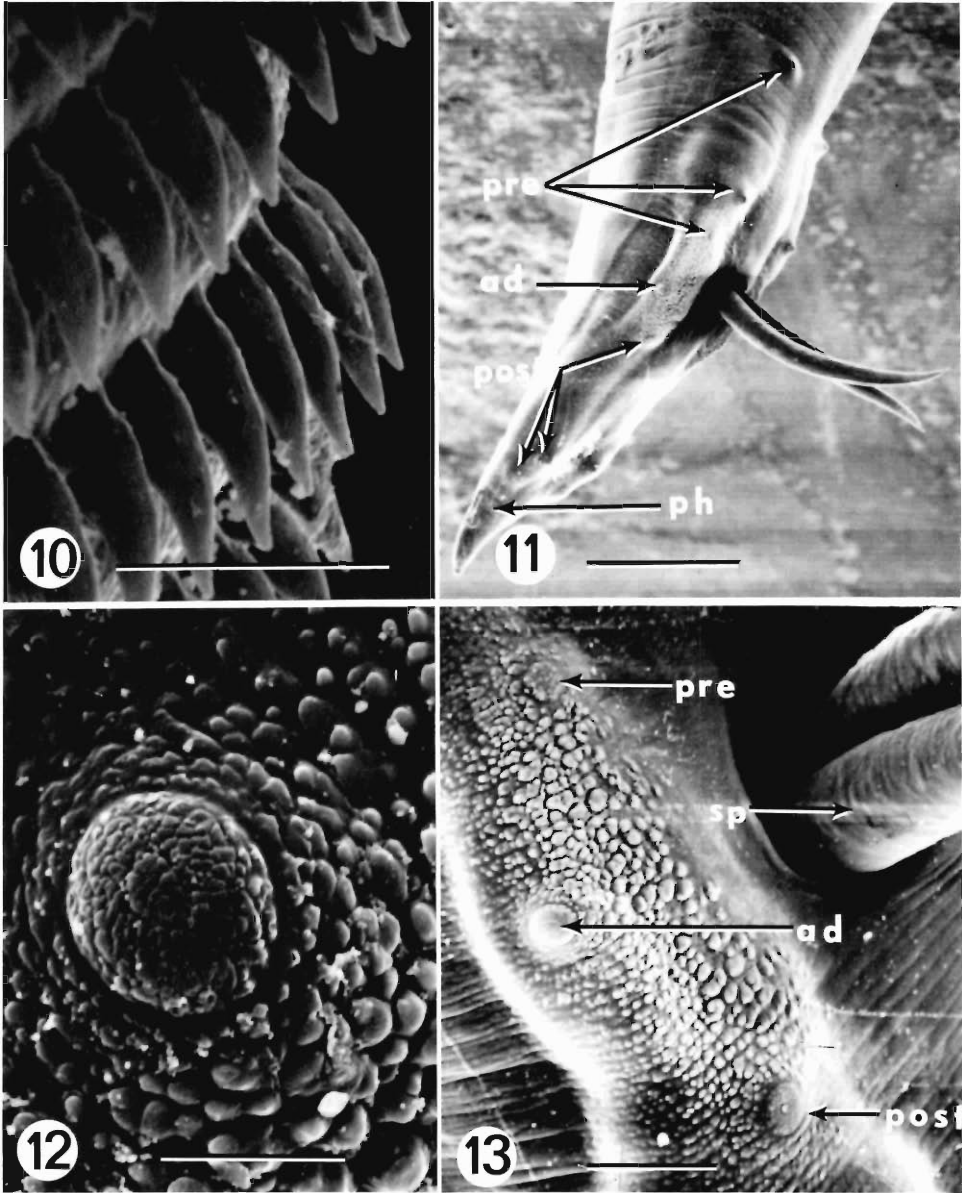
Specimens selected for scanning electron microscopy (SEM) were dehydrated, critical-point dried either in liquid carbon dioxide or in Freon 13, with Freon 113 as the transition fluid, mounted on specimen stubs, coated with 200–300 Å of gold palladium, and examined with a scanning electron microscope at 10 kV.

***Echinocephalus overstreeti* sp. n.
(Figs. 1–16)**

GENERAL: Body unarmed, reaching greatest width at posterior $\frac{1}{3}$ of body. Pseudolabia oriented dorsoventrally, equal in size, wider than long; 2 in number, each with 2 lateral double papillae and 1 medial amphid on surface; internal pulp pedunculate, trilobed. Cuticle with inconspicuous annulations. Cephalic bulb armed with spines in transverse rows; rows slightly more compact near anterior end of bulb with maximal separation near midbulb; some rows not continuous; spines uncinuate, larger at posterior of bulb; not overlapping adjacent rows; and bulb containing 4 ballonets. Cuticular collar present. Cervical sacs extending from ballonets just anterior of muscular-glandular junction of esophagus, reaching greatest width near posterior extremity, 4 in number. Esophagus 11–19% of total body length, divided near midpoint into anterior muscular and posterior glandular regions. Nerve ring located at anterior 12–17% of esophagus. Tail conical, with blunt tip; tip without ornamentation.

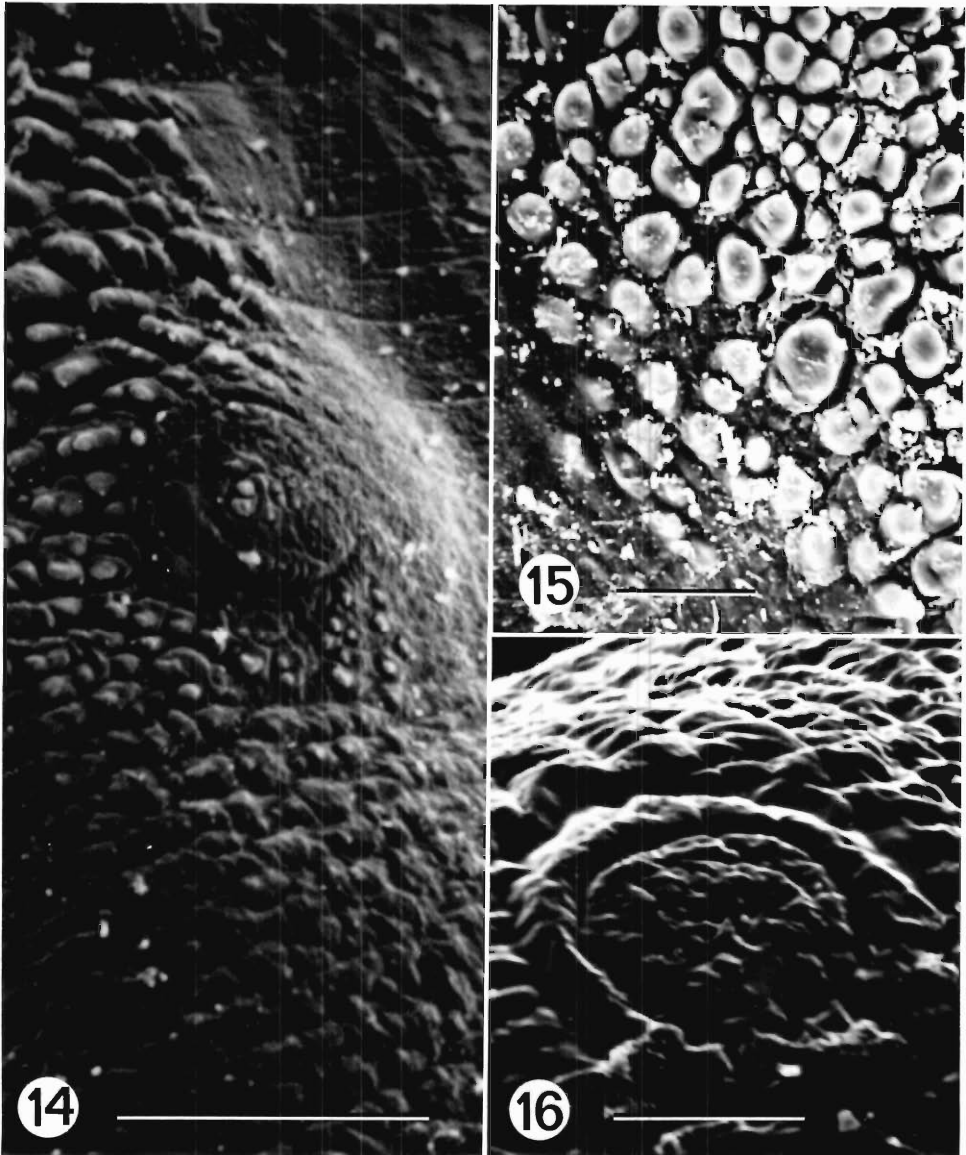


Figures 1-9. *Echinocephalus overstreeti*. 1. Anterior end, showing relationship between muscular esophagus and glandular esophagus; arrow indicates junction of parts. 2. En face. 3. Anterior extremity, showing cephalic bulb and rows of spines. 4. Posterior extremity of female tail, lateral view. 5. Lateral view of lips. 6. Dorsal view of lip. 7. Posterior end of female tail, showing opening of vulva, lateral view. 8. Posterior end of male, showing spicules, gubernaculum, caudal papillae, and rugose area, lateral view. 9. Posterior end of male, showing caudal papillae, rugose area, and gubernaculum, ventral view. Spicules not illustrated.



Figures 10-13. Scanning electron photomicrographs of male specimens of *Echinocephalus overstreeti*. 10. Cephalic spines at middle of bulb, lateral view. Bar = 10 μ m. 11. Posterior end, showing preanal (pre), adanal (ad), and postanal papillae (post), phasmid (ph), spicules, and rugose area. Bar = 200 μ m. 12. Adanal papilla surrounded by protuberances. Bar = 10 μ m. 13. Posterolateral region, showing rugose area, preanal (pre), adanal (ad), and postanal (post) papillae, and spicules (sp). Bar = 40 μ m.

MALE (based on 10 whole specimens): Body 21-30 mm long by 0.5-0.9 mm wide at greatest width; ratio of greatest width to length 1:29-58. Pseudolabia 140-220 long by 190-310 wide. Cephalic bulb 330-400 long by 450-640 wide, with 31-43 rows of cephalic spines; spines 4-11 long. Nerve ring 480-720 from anterior



Figures 14–16. Scanning electron photomicrographs of male specimen of *Echinocephalus overstreeti*. 14. Area surrounding third postanal papilla; note smooth ventral surface (upper right corner) and gradual lessening of cuticular protuberances as approaching posterior extremity (from top to bottom). Bar = 20 μm . 15. Cobblestone-like projections at lateral margin of rugose area. Bar = 10 μm . 16. First postanal papilla. Bar = 5 μm .

extremity, 40–80 in breadth. Cervical sacs 1.8–2.6 mm long by 60–140 wide at greatest width. Esophagus 11–16% of total body length, 3.2–4.7 mm long; muscular esophagus 1.0–2.0 mm long by 150–300 wide; glandular esophagus 1.2–3.2 mm long by 300–400 wide; ratio of glandular esophageal to muscular esophageal lengths 1:0.4–0.7. Spicules similar, unequal in length, 2–5% of total body length;

right spicule 0.7–1.4 mm long by 30–45 wide, longer than left spicule in 7 of 10 specimens; left spicule 0.6–1.0 mm long by 30–45 wide; spicule ratio 1:0.9–1.5. Gubernaculum V-shaped, 63–101 long. Caudal papillae 7 pairs; preanal pairs 3, decreasing in size as approaching anus; postanal pairs 3, with first pair from posterior extremity most lateral, with second pair largest, with third pair immediately posterior to anus; adanal pair 1. Medioventral preanal organ present. Rugose areas lateral to cloaca, unequal in size, 180–250 long, incorporating first preanal, adanal, and third postanal papillae. Modified annules on ventral surface lacking. Phasmids lateral, paired, near end of tail. Tail 0.4–0.7 mm long.

FEMALE (based on 10 mature specimens): Body 15–30 mm long by 0.8–1.0 mm wide at greatest width; ratio of greatest width to length 1:27–31. Pseudolabia 130–220 long by 204–280 wide. Cephalic bulb 310–510 long by 470–666 wide, with 33–39 rows of spines; spines 2–11 long. Nerve ring 590–660 from anterior extremity, 51–112 in breadth. Cervical sacs 2.0–2.8 mm long by 70–246 at greatest width. Esophagus 14–19% of total body length, 1.6–5.0 mm long; muscular esophagus 2.1–3.0 wide; glandular esophagus 1.3–1.9 mm long by 255–410 wide; ratio of glandular esophageal to muscular esophageal lengths 1:1.0–2.3. Vulval opening 0.9–1.2 mm or 1–5% of total body length from posterior extremity. Uterus didelphic, prodelphic. Eggs with smooth, thin shell; elliptical; 20–40 long by 10–30 wide. Tail 0.7–1.0 mm.

TYPE HOST: *Taeniura melanopilos* Bleeker (Dasyatidae); BPBM No. 10861.

SITE OF INFECTION: Free in spiral valve.

LOCALITY: Marquesas Islands, Huka, Motu Takatai, south side in cave 100 ft.

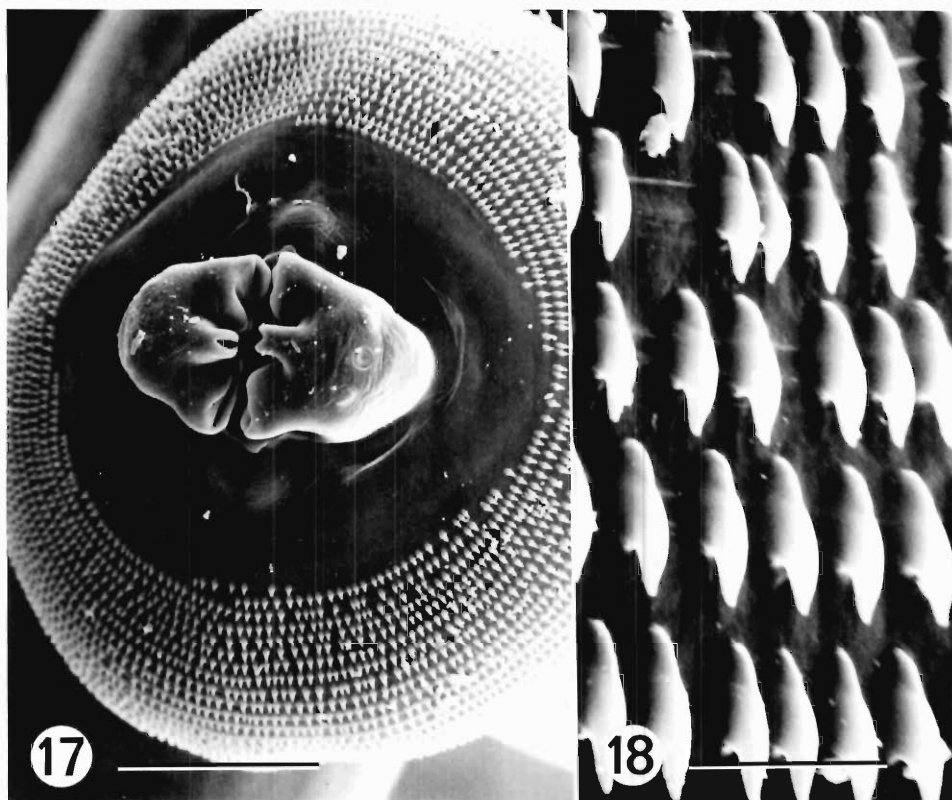
SPECIMENS DEPOSITED: Holotype, male, USNM Helm. Coll. No. 77382; allotype female, No. 77383; paratypes, No. 77384 (pair), BPBM No. 1982.531 (pair).

ETYMOLOGY: This species is named in honor of Dr. Robin M. Overstreet at the Gulf Coast Research Laboratory, Mississippi, in recognition of his considerable contributions to our knowledge of parasitology.

Remarks

Echinocephalus overstreeti differs from all known species in the genus *Echinocephalus* by having three preanal, one adanal, and three postanal pairs of papillae. It is most similar to *E. sinensis* Ko, 1975 in possessing rugose areas near the cloaca of male. On *E. overstreeti*, these areas are only lateral to the cloaca. Figures 11 and 14 show the smooth cuticle on the ventral surface that separates both rugose areas. On *E. sinensis*, however, the ventral surface between the rugose areas is not smooth (Figs. 19, 20). Moreover, *E. overstreeti* differs from *E. sinensis* in the number of cephalic spines, 31–43 rather than 26–29; in the number of pairs of adanal and postanal papillae, one compared with zero, and three rather than four, respectively; and in lacking ventral crests on the posterior of males.

Utilizing the cladogram of Deardorff et al. (1981), which depicts genealogical relationships of members of the genus *Echinocephalus*, *E. overstreeti* is part of the monophyletic group of *sinensis-dailey-daizi-pseudouncinatus*. *Echinocephalus sinensis* is unique in the sense that its known molluscan host, *Crassostrea gigas* Thunberg, is located in a brackish habitat. The biological relationships of these echinocephalids still require further elucidation. Of this group, only *E. pseudouncinatus* Millemann, 1963 was originally described as lacking a gubernaculum (Millemann, 1963). Although Deardorff et al. (1981) stated that Ko (1975) also



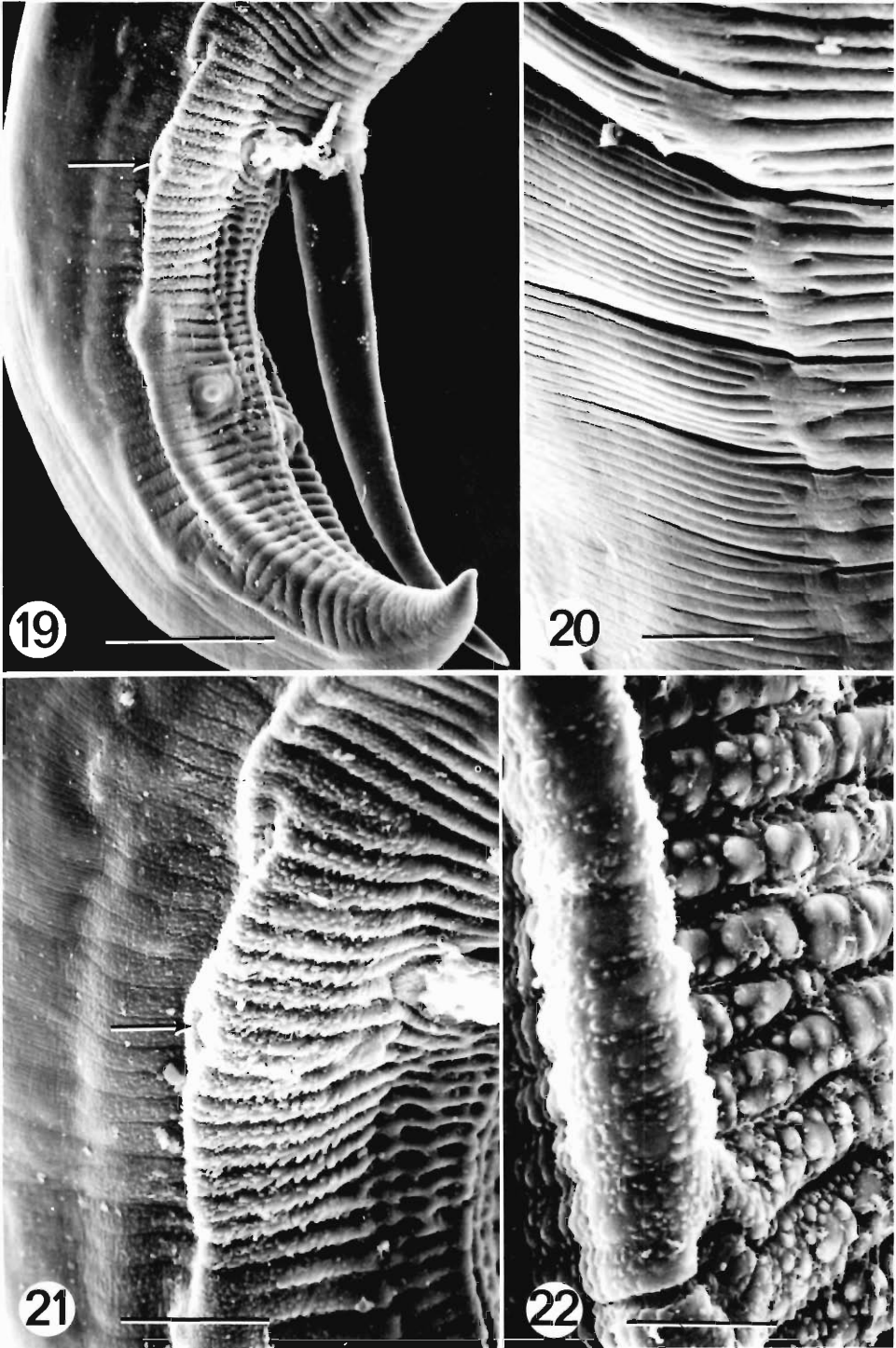
Figures 17, 18. Scanning electron photomicrographs of male *Echinocephalus sinensis*. 17. En face, showing pseudolabia with narrower medial lobes. Bar = 100 μ m. 18. Cephalic spines, showing the first five rows. Bar = 10 μ m.

reported the absence of the structure on *E. pseudouncinatus*, actually Ko (1975) reported Millemann's data (1951, 1963) and questioned the report by Millemann regarding the absence of the gubernaculum. Deardorff et al. (1981) examined *E. pseudouncinatus* and verified the presence of this structure.

***Echinocephalus sinensis* Ko, 1975**
(Figs. 17–22)

Ko (1975) described *E. sinensis* from *Aetobatus flagellum* Bloch and Schneider in Deep Bay, Hong Kong, and later Ko et al. (1980) studied the cephalic anatomy of this echinocephalid. We re-examined the holotype (male, USNM No. 73657) and noted a discrepancy in the number of caudal papillae originally reported. Ko earlier reported seven pairs of caudal papillae composed two preanal and five

Figures 19–22. Scanning electron photomicrographs of male specimen of *Echinocephalus sinensis*. 19. Posterior end of tail, showing caudal papillae, rugose area, and spicule; arrow indicates postanal papilla used for orientation in Figure 21. Bar = 100 μ m. 20. Junction of dorsal and ventral cuticle near posterior extremity. Bar = 100 μ m. 21. Lateral region of rugose area; arrow indicates postanal



papilla on Figure 19. Bar = 40 μ m. 22. Rugose area close to cloaca consisting of irregularly shaped papillary and knoblike structures. Bar = 20 μ m.

postanal papillae; the holotype, however, possesses three preanal and four postanal papillae. Ko's illustration (1975:Fig. 4) correctly locates the position of all seven pairs of caudal papillae. Apparently, the opening of the cloaca was incorrectly located, leading to the difficulty of assessing the papillae. The first pair of preanal papillae is located directly above and lateral to the opening of the cloaca. We examined 10 additional paratypes of *E. sinensis* and verified the presence and location of this papillar pair. *Echinocephalus sinensis*, even with the change in the number of preanal and postanal papillae, may be easily distinguished from all other species in the genus and remains a valid species.

The body cuticle of *E. sinensis* at the posterior extremity of males is highly ornate when compared with *E. overstreeti*. Modified ventral crests, not reported in the original description, are present on the surface of the holotype above the opening of the cloaca. The rugose areas, located on the ventrolateral regions adjacent to the cloaca, on *E. sinensis* consist of irregularly shaped papillary and knoblike structures (Fig. 22), whereas on *E. overstreeti* the projections are smooth and give a "cobblestone" appearance (Fig. 15). The irregular protuberances are situated on transverse rows of ridges. These rows of ridges are located from the first pair of preanal papillae to nearly the tip of the tail and extend across the ventral surface. Cuticular projections are lacking on the transverse ridges directly below the cloaca (Figs. 19, 21, 22). The cuticle on the ventromedial region behind the cloaca appears in lattice form. A pair of medial, longitudinal ridges, which also lack ornamentation, extends from immediately below the cloaca to nearly the posterior extremity (Fig. 19). The ornate ventral cuticle terminates abruptly along the lateral aspect of the worm (Figs. 19–22). The dorsal tail cuticle has narrower striations as compared to those of the ventral region, forming a marked junction laterally (Fig. 20).

Acknowledgments

We acknowledge the following persons: J. R. Lichtenfels at the U.S. National Museum Helminthological Collection (Beltsville, Maryland) for loaning specimens; J. E. Randall and W. Steffan, both at the B. P. Bishop Museum (Honolulu), for permission to remove worms from stomachs of several museum hosts and for use of drawing equipment, respectively; and J. R. Yates III at the University of Hawaii and R. L. Edmonson at Leahi Hospital for assistance at the SEM facility. This study was supported, in part, by Contract No. 223-80-2295 from the Food and Drug Administration and by the U.H. Sea Grant College Program under Institutional Grant No. NA81AA-D-00070 from NOAA, Office of Sea Grant, Department of Commerce. This constitutes Sea Grant Publication UNIHI-SEA-GRANT-JC-84-04.

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EMOP IV 1984
FOURTH EUROPEAN MULTICOLLOQUIUM OF
PARASITOLOGY

IZMIR, TURKEY
October 14–19, 1984

The Fourth European Multicolloquium of Parasitology will be held in Izmir, Turkey, on October 14–19, 1984. Preliminary plans and first announcement were recently issued by the organizing committee (Prof. Sevkett Yasarol, President; Prof. M. Ali Ozcel, Vice President).

The meeting will be held at the Ege University Cultural Center in downtown Izmir. The scientific program will include plenary sessions, symposia, workshops, and free-communication (oral and poster) sessions. Topics will include but are not limited to the following:

1. Systematics and Evolution
2. Morphology and Ultrastructure
3. Biochemistry and Physiology
4. Ecology and Epidemiology
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6. Clinical Parasitology
7. Control and Prevention

If you are interested, write to Dr. Emel Tumbay, M.D., Secretary General of EMOP IV 1984, P.K. 81 (post office box number), Bornova-Izmir, Turkey.

***Ichthyocephalus seymouri* sp. n. and a Redescription of
Ichthyocephalus ichthyocephalus Artigas, 1926
(Nematoda: Rhigonematidae) from
Diplopods in Brazil**

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ABSTRACT: *Ichthyocephalus seymouri* sp. n. (Ichthyocephalinae; Rhigonematidae) is described from *Rhinocricus nattereri* from Bahia, Brazil. The new species is most similar to *I. ichthyocephalus* Artigas, 1926 in that there is a pronounced sexual dimorphism with respect to size. *Ichthyocephalus seymouri* is easily distinguished from the latter in that the esophageal corpus of the male is elongate (as opposed to subspherical), by the shape of the left spicule, by the number of caudal papillae, and by the fact that spines on the body cuticle are longest in the midbody region. *Ichthyocephalus ichthyocephalus*, type species of the genus, is redescribed on the basis of neotypes designated by Travassos and Kloss (1958) from *Rhinocricus padbergi* from Manguinhos, Brazil.

Artigas (1926) proposed *Ichthyocephalus*, with *I. ichthyocephalus* as type species, for nematode parasites of diplopods characterized by a laterally elongate mouth opening surrounded by dorsal and ventral lips and by a large, ovoid, muscular esophageal corpus. Since then, several species have been added to the genus and the genus has been reviewed twice (Travassos and Kloss, 1958b, 1965). Despite this, the structure and function of the cephalic extremity and esophageal corpus remain unclear.

During an examination of diplopods in the Paris Museum collection (Muséum national d'Histoire naturelle; Laboratoire des Arthropodes), a new species of *Ichthyocephalus* was discovered. This species is described here and care is taken to clarify cephalic morphology. In addition, *Ichthyocephalus ichthyocephalus* is redescribed.

Materials and Methods

The specimen of *Rhinocricus nattereri*, from the Muséum diplopod collection (Jar H79: tube DG LXI bis), had been stored in 70% ethanol. Nematodes were stored in 70% ethanol before being cleared and examined in lactophenol.

Dr. Delir Correa Gomez (Instituto Oswaldo Cruz, Rio de Janeiro) kindly loaned neotypes of *I. ichthyocephalus*.

Results

1. *Ichthyocephalus seymouri* sp. n.

MATERIAL EXAMINED: One entire male (holotype), one entire female (allotype) and several pieces of females collected from *Rhinocricus nattereri* (Rhinocricidae; Diplopoda) from Bahia, Brazil. Nematodes are stored in the museum helminth collection (Laboratoire des Vers: RA 28).

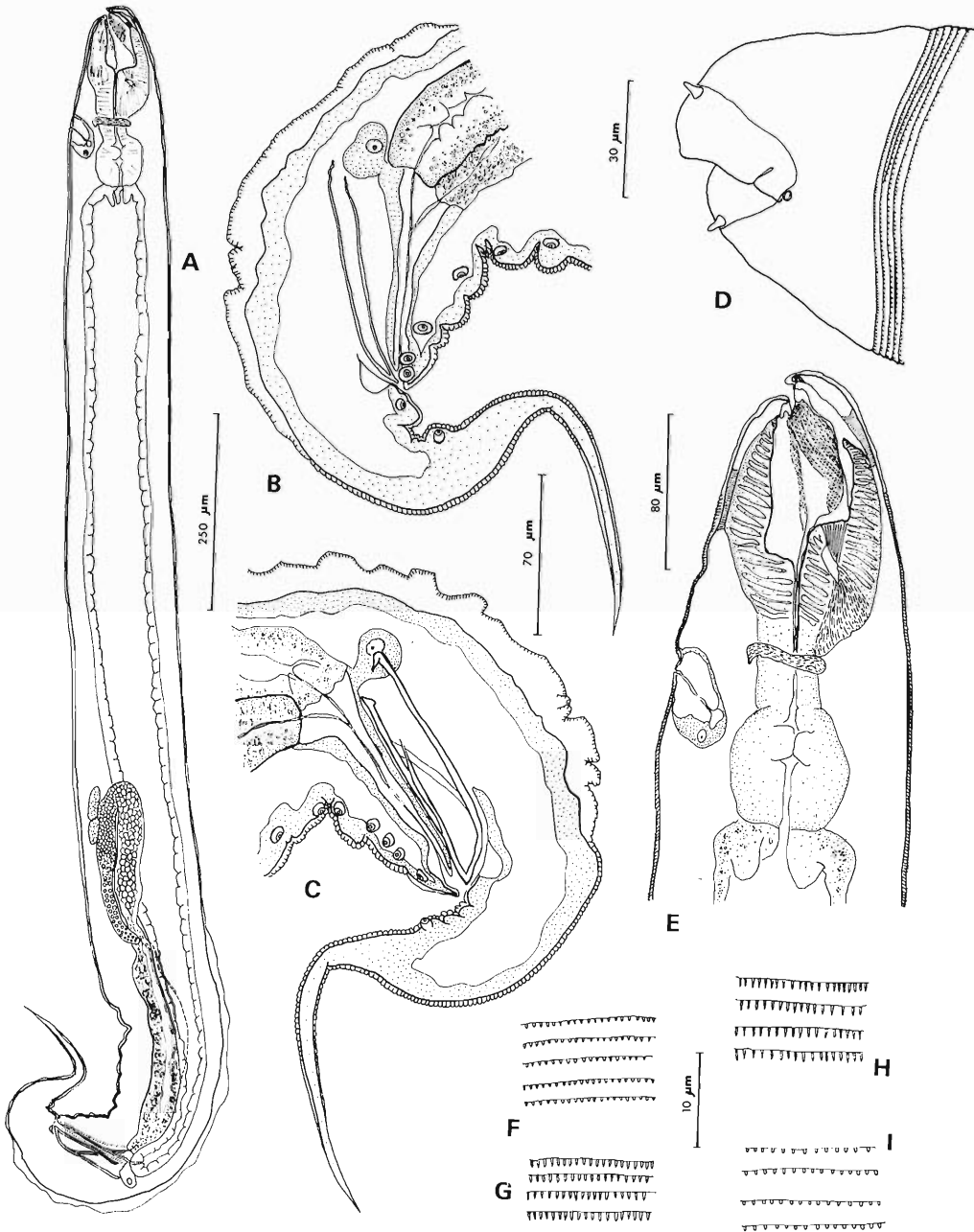


Figure 1. *Ichthyocephalus seymouri* sp. n. A. Entire male, lateral view. B. Caudal end of male, right side. C. Caudal end of male, left side. D. Cephalic extremity of male, lateral view. E. Esophageal region of male, lateral view. F-I. Cuticular ornamentation. F. Male, near level of esophageal corpus. G. Male, near midbody. H. Female, at level of esophageal corpus. I. Female, near level of anterior flexure of reproductive tract. (A, scale = 250 μ m; B and C, scale = 70 μ m; D, scale = 30 μ m; E, scale = 80 μ m; F-I, scale = 10 μ m.)

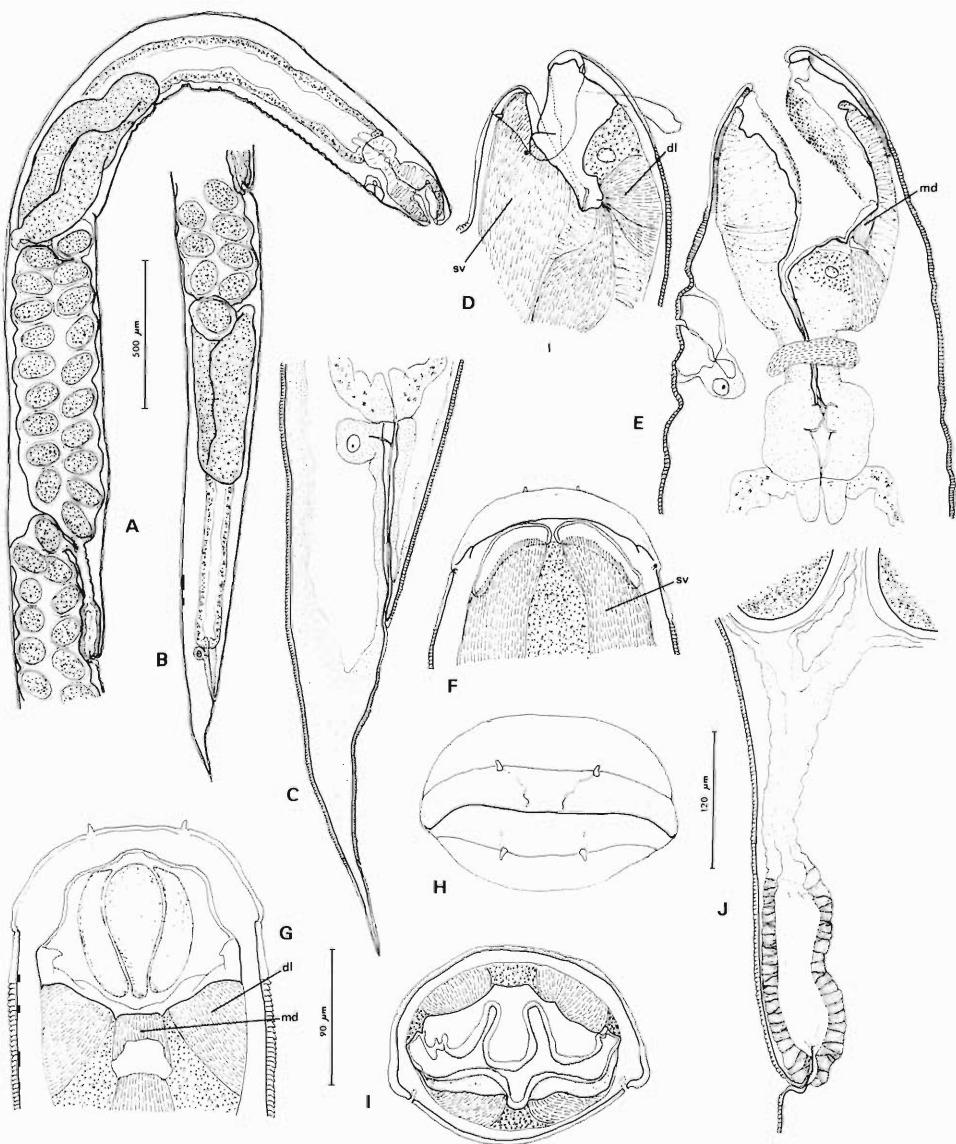


Figure 2. *Ichthyocephalus seymouri* sp. n., female. A and B. Anterior and posterior halves of worm, lateral view. C. Caudal end, lateral view. D. Cephalic extremity, lateral view, showing subventral, lateral, and dorsolateral jaw muscles. E. Cephalic extremity, lateral view, optical section, showing median dorsal (md) muscle. F. Cephalic extremity, ventral view, showing subventral muscles (sv). G. Cephalic extremity, dorsal view, showing dorsal plate of rasplike cuticle and dorsolateral (dl) and median dorsal (md) muscles. H. Superficial apical view. I. Section through middle of esophageal corpus, apical view. (A and B, scale = 500 μ m; C, scale = 120 μ m; D-I, scale = 90 μ m.)

Description

GENERAL: Pronounced sexual dimorphism with respect to size. Oral opening a lateral slit bordered by a large dorsal labium overhanging ventral labium. Amphids at corners of mouth. Four conical submedian cephalic papillae present. Inconspicuous nerve present, leading toward edge of mouth opening from each papilla.

Lumen in anterior half of esophageal corpus laterally elongate and lined by thick dorsal and ventral layers of cuticle. Dorsal layer with 3 and ventral layer with 2 longitudinal ridgelike swellings bearing many tiny toothlike formations giving cuticular lining a rasplike appearance. Corpus firmly attached to body cuticle anteriorly.

Muscles of corpus consisting of: 1 pair lateral (Fig. 2D, l) and 1 pair dorsolateral (Fig. 2D and G, dl), leading from lateral surface of corpus to lateral edge of cuticle lining lumen; median dorsal (Fig. 2E and G, md), leading posteriorly to cuticular plate, which is in turn connected to posterodorsal edge of corpus by muscle; 1 pair subventral (Fig. 2D and F, sv) running along ventral side of corpus.

Excretory system with prominent pore, vesicular terminal duct, and ventral transverse canal leading to anterior and posterior lateral canal on either side. Nine (3 dorsal and 6 subventral) large arcade cells present just posterior to corpus, much more prominent in female than in male. Body cuticle with prominent striations 2–3 μm apart, beginning about 60 μm from anterior extremity. Posterior edge of each stria with tiny cuticular spines.

MALE: Cuticular spines gradually increasing in size posteriorly, reaching maximum size near midbody, thereafter becoming smaller and less regular, and disappearing near level of vas deferens. Single pair of coelomocytes present, near flexure of testis.

Eight pairs of caudal papillae present, all subventral: 6 pairs preanal, 1 pair adanal, and 1 pair postanal. Two dissimilar, subequal spicules present. Left spicule robust, well cuticularized; right spicule slender and more delicate.

FEMALE: Cuticular spines on body cuticle gradually becoming smaller posteriorly, but present to near end of tail.

Vulva in posterior half of body, leading anteriorly to muscular vagina. Muscular layer of vagina becoming thinner anteriorly and disappearing before vagina divides into anterior and posterior uterine branches. Eggs with smooth shell 10–12 μm thick, containing embryo in early stage of development.

DIMENSIONS: MALE HOLOTYPE: Length 1.95 mm. Maximum width 167 μm near midbody. Esophageal corpus 144 μm and isthmus 30 μm long. Bulb 64 μm long and 60 μm wide. Nerve ring 152 μm and excretory pore 163 μm from anterior extremity. Left spicule 111 μm , right spicule 108 μm and tail 239 μm long.

FEMALE ALLOTYPE: Length 5.53 mm. Maximum width 338 μm near midbody. Esophageal corpus 253 μm and isthmus 48 μm long. Bulb 66 μm long and 82 μm wide. Nerve ring 273 μm , excretory pore 245 μm , and vulva 3.44 mm from anterior extremity. Tail 292 μm long. Eggs 144–153 μm long and 103–110 μm wide.

Discussion

This species is most similar to *I. ichthyocephalus* (see below). Both species are characterized by a pronounced sexual dimorphism with respect to size. *Ichthyocephalus seymouri* sp. n. is easily distinguished from *I. ichthyocephalus* in having an elongate as opposed to subspherical corpus in the male, by the shape of the left spicule, and by the number of caudal papillae. In addition, in males of *I. seymouri*, spines on the body cuticle are longest near midbody, whereas those of *I. ichthyocephalus* are longest in the most anterior spine rows. Finally, the tail of the female is shorter and the eggs are larger in *I. seymouri*.

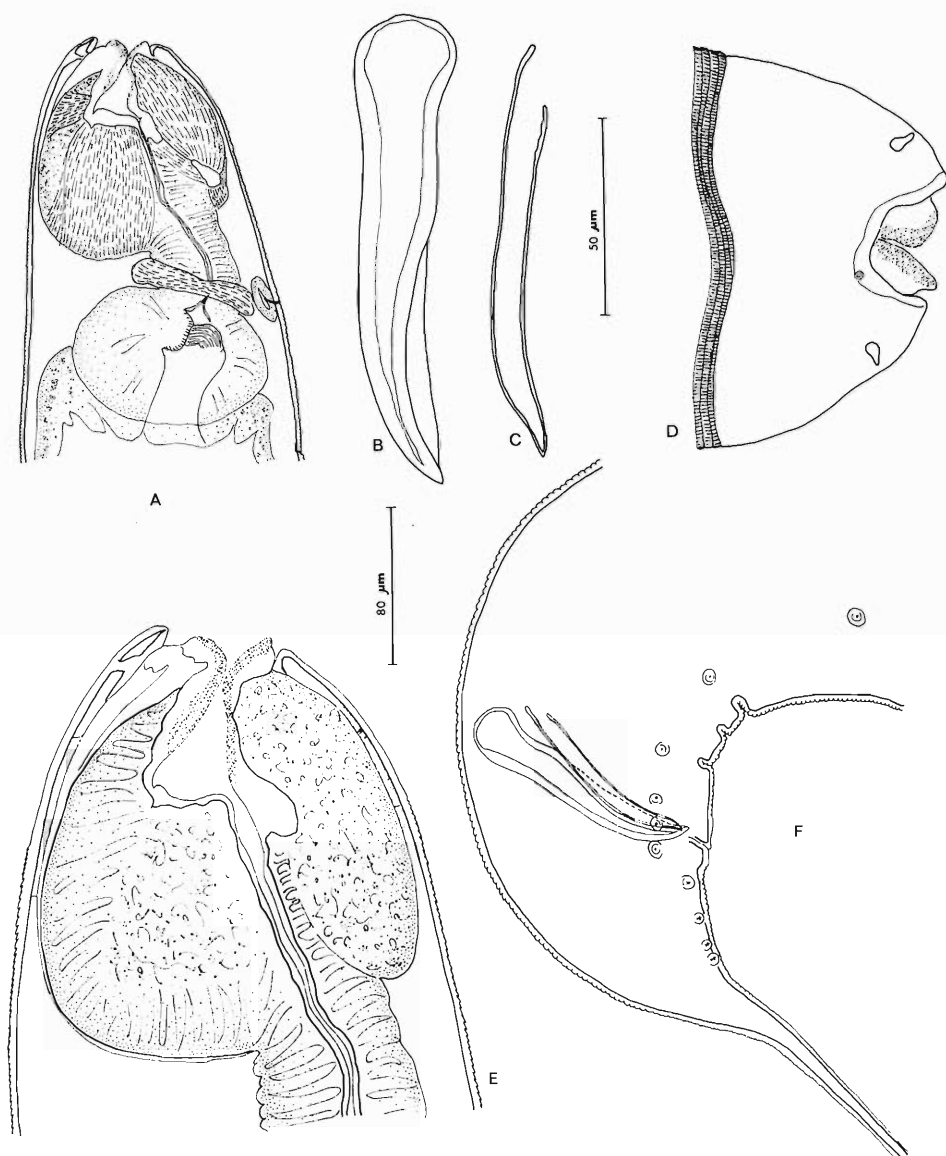


Figure 3. *Ichthyocephalus ichthyocephalus* Artigas, 1926, male. A. Esophageal region, lateral view. B and C. Left and right spicules. D. Cephalic extremity, lateral view. E. Esophageal corpus, lateral view. F. Caudal extremity, lateral view. (A and F, scale = 80 μ m; B-E, scale = 50 μ m.)

The valved bulb at the base of the esophagus in rhigonematids (as well as oxyuroids and cosmocercoids) has a grinding as well as a pumping function. In *Ichthyocephalus*, the grinding function is probably largely taken over by the esophageal corpus, which consists of powerful muscles operating dorsal and ventral rasplike cuticular plates. The esophageal bulb is reduced, although it presumably retains its pumping function.

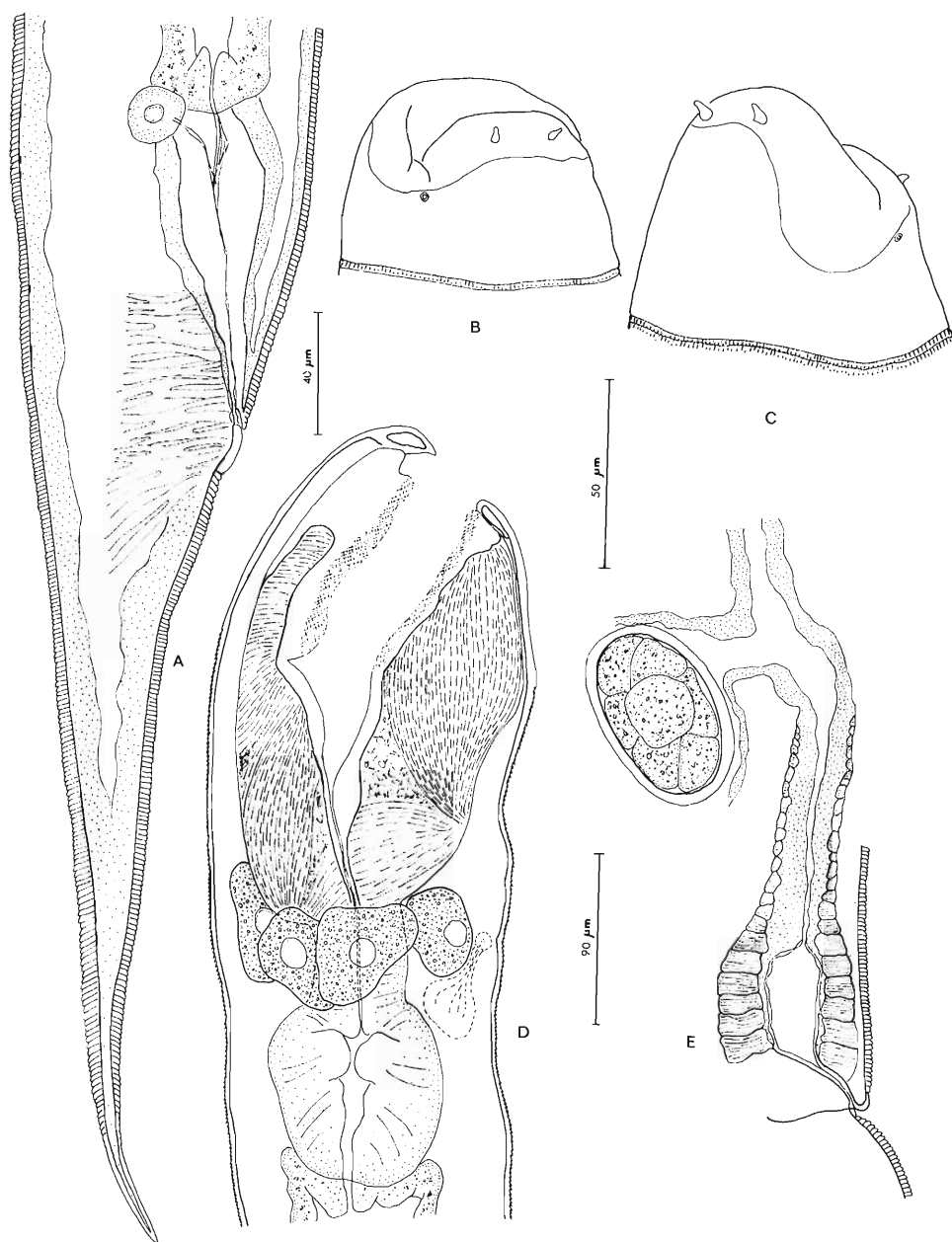


Figure 4. *Ichthycephalus ichthycephalus* Artigas, 1926, female. A. Caudal end, lateral view. B and C. Cephalic extremities of the two females. B. Subventral view. C. Subdorsal view. D. Esophageal region, sublateral view; note prominent arcade cells (ar) and excretory system (broken lines). E. Vulva and vagina, lateral view. (A, scale = 40 μ m; B, C, and E, scale = 50 μ m; D, scale = 90 μ m.)

2. *Ichthycephalus ichthycephalus* Artigas, 1926

MATERIAL EXAMINED: Neotypes: 2 females (No. 22965) and 1 male (No. 22966) in permanent mounts, stored in the helminth collection of the Instituto Oswaldo Cruz, Rio de Janeiro.

Description

MALE: Length 2.33 mm. Maximum width 213 μm near midbody. Esophageal corpus 107 μm and isthmus 28 μm long. Bulb 54 μm long and 88 μm wide. Nerve ring and excretory pore 120 μm from anterior extremity. Right spicule 103 μm , left spicule 120 μm , and tail 230 μm long.

Cuticular spines present, longest anteriorly, disappearing near level of base of esophagus.

Rasplike plates of cuticle lining esophageal corpus projecting anteriorly and protruding slightly from mouth opening. Esophageal corpus subspherical and esophageal bulb much wider than long.

Spicules dissimilar and subequal. Right spicule delicate and slender, resting in spoonlike depression on inside surface of left spicule. Ten pairs of caudal papillae present, all subventral: 5 pairs preanal and 5 pairs postanal.

FEMALE (2 specimens): Length 6.68 and 6.89 mm. Esophageal corpus 246 and 265 μm , and isthmus 45 and 48 μm long. Bulb 103 and 108 μm long, and 88 and 117 μm wide. Nerve ring 266 and 285 μm , excretory pore 257 and 304 μm , and vulva 3.88 and 4.29 mm from anterior extremity. Tail 660 and 510 μm long. Eggs 126–139 μm long and 75–98 μm wide.

Discussion

Artigas (1926) described this species on the basis of female specimens from an unidentified diplopod from Remedios Province (State of São Paulo) and Manguinhos (Federal District), Brazil. In 1930, he redescribed the material in his thesis. In a revision of the genus, Travassos and Kloss (1958a) noted that the types had been lost; they designated neotypes from *Rhinocricus padbergi* from Manguinhos and redescribed the species. In 1965, they redescribed the species from several other Brazilian localities.

In addition to species described here, *Ichthyocephalus* contains the following: *I. ichthyocephaloides* Dollfus, 1952 from *Rhinocricus* sp. from the State of São Paulo, Brazil; *I. egleri* Travassos and Kloss, 1958b from *R. insulsus* from the State of Pará, Brazil; *I. antenori* Travassos and Kloss, 1959 from *R. urucumai* from the State of Goiás, Brazil.

Paraichthyocephalus Travassos and Kloss, 1958a resembles *Ichthyocephalus* in cephalic structure, but differs in that the isthmus is longer than the esophageal bulb and in that males have caudal alae and similar spicules. There are three species, all from Brazil, namely, *P. artigasi* (Almeida, 1933) (type species) from *R. cachoeirensis*, *P. almeidai* (Dollfus, 1952) from *Hemigymnostreptus* sp., and *P. hirsutus* Travassos and Kloss, 1965 from *Spirostreptus* sp.

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***Paratrophurus acristylus* sp. n. and
Tylenchorhynchus graciliformis sp. n. (Nematoda: Tylenchida)
from Wheat Fields in Libya**

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ABSTRACT: *Paratrophurus acristylus* sp. n. and *Tylenchorhynchus graciliformis* sp. n. are described from wheat fields in Libya. *Paratrophurus acristylus* has a slender (18–21- μ m-long) spear with small basal knobs 2.5–3.2 μ m across, a subclavate female tail with terminal 21–28% hyaline, a postanal intestinal sac filling about three-quarters of tail cavity, and 22.5–23- μ m-long spicules. *Tylenchorhynchus graciliformis* is slender ($a = 40$ –49 in females, 45–52 in males) and has a knoblike lip region with 5–6 distinct annules, a 17–18.5- μ m-long spear with large basal knobs 4.5–5 μ m across, female tail with 16–20 annules, and spicules 19–21 μ m long.

During a 1980–1981 survey of Libyan wheat fields for plant-parasitic nematodes made by one of us (Z.A.S.), two new species belonging to the family Dolichodoridae were found. These are described here as *Paratrophurus acristylus* sp. n. and *Tylenchorhynchus graciliformis* sp. n. The nematodes were heat-killed, fixed in a 3% solution of formaldehyde, and mounted in dehydrated glycerine after processing them through warm lactophenol. All the measurements were taken from glycerine-mounted specimens.

***Paratrophurus acristylus* sp. n.
(Fig. 1A–E)**

MEASUREMENTS: Holotype ♀: $L = 0.69$ mm; $a = 33$; $b = 5.6$; $c = 18$; $c' = 2.5$; $V = 58$; spear 20 μ m. Paratypes: 6 ♀: $L = 0.58$ –0.73 (0.65) mm; $a = 31$ –38 (34); $b = 4.9$ –5.7 (5.3); $c = 17$ –20 (18.5); $c' = 2.2$ –2.8 (2.5); $V = 55$ –59 (57); spear 18–21 (20) μ m. 2 ♂: $L = 0.64$ –0.72 mm; $a = 39$ –40; $b = 5.7$ –5.9; $c = 18$ –19; $c' = 2.6$ –2.7; $T = 50$ –52; spear 18–21 μ m; spicules 22.5–23 μ m; gubernaculum 11–12 μ m.

DESCRIPTION: FEMALE: Body arcuate, cuticle thick with fine transverse striae averaging 1.2 μ m apart, abnormally thickened (8–12 μ m) on tail tip. Lateral fields with 4 smooth, equidistant incisures, $\frac{2}{5}$ – $\frac{1}{3}$ body width. Lip region continuous, anteriorly conoid rounded; framework lightly sclerotized. Spear extremely slender, sharply pointed (hence the species name); conus 52–57% (54%) of spear length; knobs minutely rounded, 2.5–3.2 μ m across. Orifice of dorsal esophageal gland 2.5–3.5 μ m behind spear base. Median esophageal bulb rounded, 11.5–12.5 μ m \times 9–10 μ m, with distinct valve plates. Basal bulb small 12–17 μ m \times 7–10 μ m, with base offset or slightly extending over intestine. Esophago-intestinal valve large, rounded. Distance from anterior end of body to center of median bulb 60–68 (65) μ m or 53–54% of esophageal length. Excretory pore 2–3 annules behind hemizonid, 90–110 (102) μ m from anterior end. Vulva transverse. Vagina about $\frac{1}{2}$ body width long. Both branches of reproductive organs well developed, symmetrical, outstretched. Spermatheca with sperm in most females. Ovaries with oocytes in a row. Rectum about $\frac{1}{2}$ anal body width long. Postanal intestinal sac 17–23 μ m long, extending into tail cavity for about $\frac{3}{4}$ of its length or 50–62%

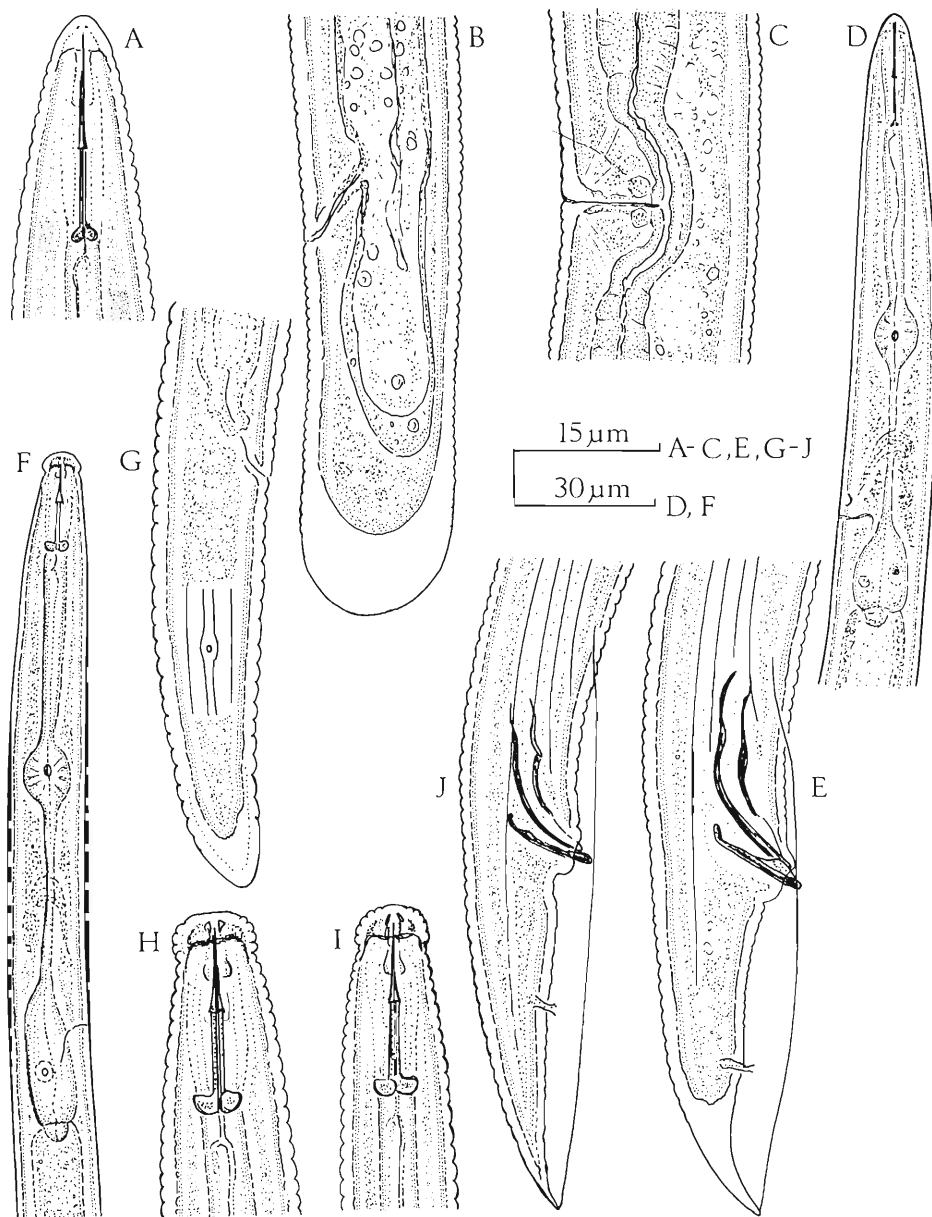


Figure 1. A-E. *Paratrophurus acristylus* sp. n. F-J. *Tylenchorhynchus graciliformis* sp. n. A and H. Head ends, female. B and G. Tail ends, female. C. Vulval region. D and F. Esophageal regions, female. E and J. Tail ends, male. I. Head end, male.

(54%) of tail length. Tail cylindroid at first, then slightly subclavate terminally where annules are indistinct; hyaline portion 21–28% of tail length; phasmids at about middle of tail.

MALE: Body C-shaped. Lip region, spear, and esophagus as in female. Testis outstretched. Spicules arcuate, prominently flanged distally. Gubernaculum large,

protrusible, with proximal end directed forward. Bursa enveloping tail, with smooth margins. Phasmids at middle of tail, extending into bursa.

TYPE HOST AND LOCALITY: Wheat (*Triticum aestivum* L.), Al'Azizia, Libya.

TYPE SPECIMENS: Holotype ♀ and 4 ♀ paratypes at C.I.P., St. Albans, England; 2 ♀ paratypes each at USDA Nematode Collection, Beltsville, Maryland, and Indian Agricultural Research Institute, New Delhi, India.

RELATIONSHIP: *Paratrophurus acristylus* sp. n. differs from *P. loofi* Arias, 1970 in having a more slender spear, a longer postanal intestinal sac, and shorter spicules (intestinal sac very slightly or not extending into tail cavity, spicules 26–27 µm long in *P. loofi*). It can be differentiated from *P. kenanae* Decker and El-Amin, 1978 by its slender spear having smaller basal knobs (4.5–5 µm across in latter), smaller hyaline portion of tail (12–16 µm long in latter), and a well-developed postanal intestinal sac.

***Tylenchorhynchus graciliformis* sp. n.**

(Fig. 1F–J)

MEASUREMENTS: Holotype ♀: L = 0.8 mm; a = 47; b = 5.7; c = 19; c' = 3.6; V = 22–57–19; spear 18 µm. Paratypes: 15 ♀: L = 0.67–0.83 (0.78) mm; a = 40–49 (45); b = 5.7–6.8 (6); c = 18–25 (20); c' = 2.3–3.7 (3); V = 53–60 (57); spear 17–18.5 (18) µm. 6 ♂: L = 0.68–0.78 (0.72) mm; a = 45–52 (50); b = 4.8–6.1 (5.3); c = 20–22 (21); c' = 2.6–3.1 (2.8); T = 40–56 (46); spear 16.5–18 (17.5) µm; spicules 19–21 (20) µm; gubernaculum 10–12 (11) µm.

DESCRIPTION: FEMALE: Body very slender (hence the species name), slightly arcuate; maximum width 15–18 µm. Cuticle coarsely annulated, annules averaging 2.1 µm wide near midbody. Lateral fields wide, about 1/3 body width, with 4 equally spaced incisures, not areolated; lip region offset knoblike, with 5 or 6 (usually 5) distinct annules, 1/3 as high as wide, framework lightly sclerotized, with basal plate conspicuously pushed into lip region cavity (Fig. 1H). Spear robust, with conus 7.5–8.5 µm, or less than 1/2 its length, and large basal knobs 4.5–5 µm across and having flattened to concave anterior surfaces. Orifice of dorsal esophageal gland 3–3.5 µm behind spear base. Excretory pore 104–122 (112) µm from anterior end, 1–3 annules behind hemizonid. Median esophageal bulb strongly muscular, 12–15 µm × 9–10 µm, at 44–48% of esophagus. Basal bulb elongate-saccate, 26–33 µm × 10–12 µm; esophago-intestinal valve large, rounded. Vulva transverse. Vagina about 1/2 body width long. Spermatheca spherical to elongate oval, with sperm in fertilized females. Ovaries outstretched with oocytes in a row. Rectum about anal body width long. Tail elongate-conoid to subcylindrical, with a smoothly rounded tip and 16–20 (18) annules ventrally. Phasmids near or just anterior to middle of tail.

MALE: Body straight to arcuate. Cuticle, lip region, spear and esophagus as described for female. Spicules cephalated, ventrally arcuate, with large distal flanges. Gubernaculum protrusible, with proximal end directed anteriorly. Bursa enveloping tail.

TYPE HOST AND LOCALITY: Wheat (*Triticum aestivum*), Tarhunah, Libya. Also collected around wheat roots in Abu Ayesha and Abu Shoeb, Libya.

TYPE SPECIMENS: Holotype ♀ and 11 ♀, 4 ♂ paratypes at C.I.P., St. Albans, England; 2 ♀, 1 ♂ paratypes each at USDA Nematode Collection, Beltsville, Maryland, and Indian Agricultural Research Institute, New Delhi, India.

RELATIONSHIP: *Tylenchorhynchus graciliformis* sp. n. comes close to *T. cylindricus* Cobb, 1913, but differs in having a smaller body, spear, and spicules, the basal plate of the labial framework conspicuously pushed into the lip region cavity, and the larger spear knobs with flattened to concave anterior surfaces. According to the redescription by Lewis and Golden (1981), *T. cylindricus* has ♀ L = 0.721–1.179 (1.009) μm ; ♀ spear 25.1–29.5 (27.4) μm long with rounded knobs sloping posteriorly; and spicules 27.7–32.7 (29.8) μm long. This new species also resembles *T. aduncus* de Guiran, 1967, *T. latus* Allen, 1955, and *T. brassicae* Siddiqi, 1961, but differs from them in having a slender body, lip region one-third as high as wide and a robust spear with large knobs 4.5–5 μm across.

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Neotropical Monogenoidea. 4.

***Linguadactyloides brinkmanni* gen. et sp. n. (Dactylogyridae: Linguadactyloidinae subfam. n.) with Observations on its Pathology in a Brazilian Freshwater Fish, *Colossoma macropomum* (Cuvier)**

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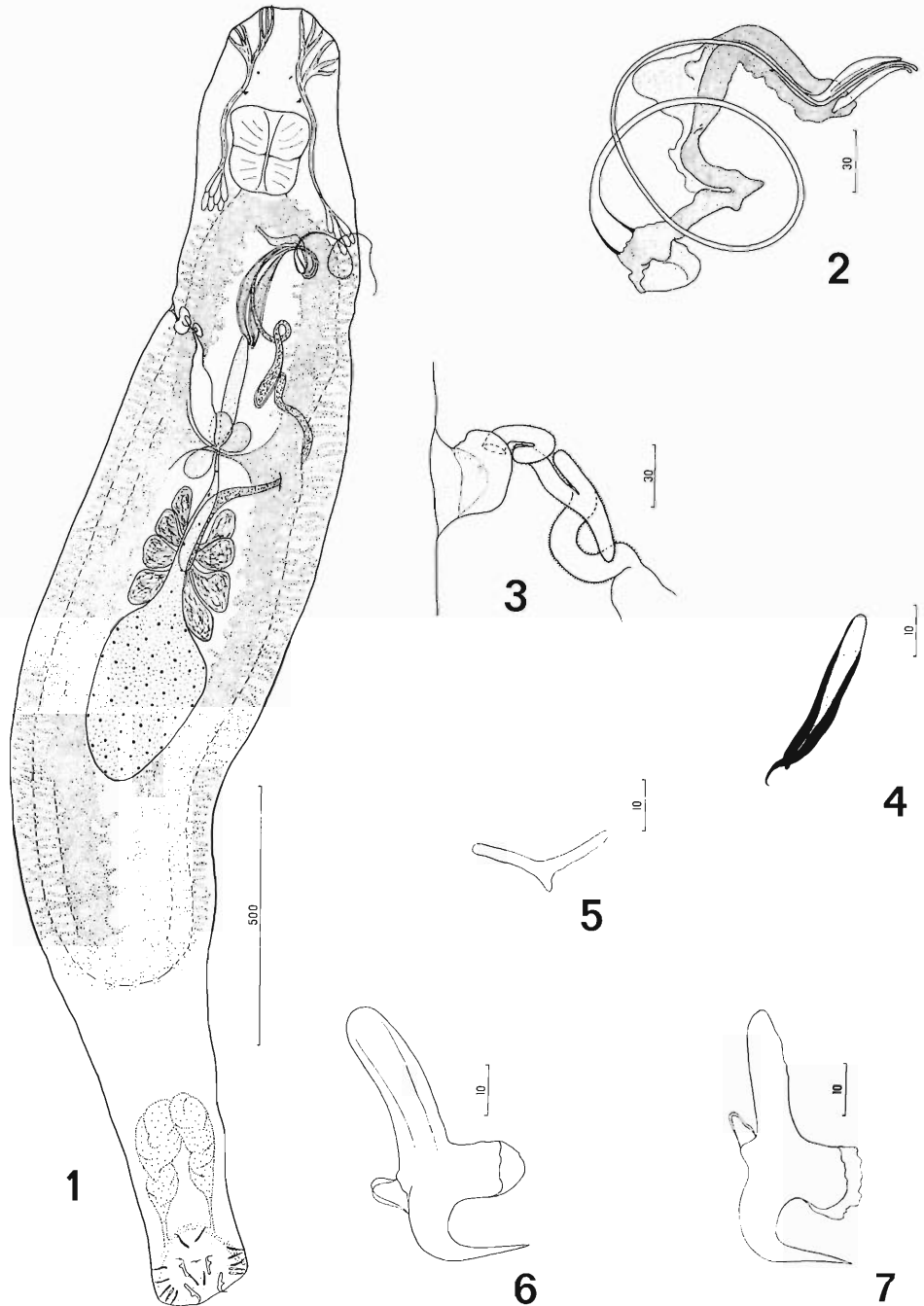
ABSTRACT: *Linguadactyloides brinkmanni* gen. et sp. n. (Monogenoidea: Dactylogyridae: Linguadactyloidinae subfam. n.) is described from the gills of the freshwater fish, *Colossoma macropomum* (Cuvier) (Characidae), from Janauacá Lake near Manaus, Amazonas, Brazil. This species is characterized by having three or four pairs of preovarian testes, three reservoir-like bodies of the female duct near the ootype, and a single haptor bar. *Linguadactyloides* gen. n. and Linguadactyloidinae subfam. n. are proposed for this species. The mode of attachment and associated pathology of the parasite on the gills of the host are presented.

Since its proposal by Brinkmann (1940), the monotypic genus *Linguadactyla* has held a rather isolated position within the Dactylogyridae. Bychowsky (1957) proposed the subfamily Linguadactylinae for this genus, based on the presence of a follicular testis (numerous testes according to Yamaguti, 1963), a single haptor bar, intestinal crura with diverticula, and a complex system of vitelline ducts with three vesicular outgrowths. Until our discovery of *Linguadactyloides brinkmanni* gen. et sp. n. from *Colossoma macropomum* (Cuvier) in Brazil (April 1979), no form apparently related to this species was known. The present study includes the description of the new species and the proposal of *Linguadactyloides* gen. n. Because some morphological features of *L. brinkmanni* preclude its inclusion in Linguadactylinae, a new subfamily, Linguadactyloidinae, of the Dactylogyridae is proposed.

We have observed that *L. brinkmanni* has attachment and feeding habits quite different from those of other Monogenoidea, which result in a high level of pathogenicity. The pathological effects of this parasite on its host are also reported herein.

Materials and Methods

Fish gills were placed in finger bowls and covered with a 1:4,000 formalin solution. After ½ hr, the gills were agitated in this liquid and then removed from the bowl. The helminths were allowed to settle to the bottom and were subsequently removed with the aid of small probes and a dissecting microscope. They were immediately fixed and stored in AFA. Some were mounted unstained in Gray and Wess' medium for study of sclerotized structures. Other specimens stained with Mayer's acid carmalum or Gomori's trichrome were used to determine internal features. Material for determining pathology was prepared using standard histologic techniques; some living specimens attached to gill filaments were studied with a dissecting microscope. Measurements, all in micrometers,



Figures 1-7. *Linguadactyloides brinkmanni* gen. et sp. n. 1. Composite illustration of whole mount (ventral). 2. Copulatory complex. 3. Vagina. 4. Hook. 5. Ventral bar. 6. Ventral anchor. 7. Dorsal anchor.

were made according to the procedures of Mizelle and Klucka (1953). Illustrations were prepared with the aid of a camera lucida or microprojector.

Linguadactyloidinae subfam. n.

DIAGNOSIS: Dactylogyroidea; Dactylogyridae. Cephalic region developed into cephalic lobes; head organs, cephalic glands present. Eyes present. Mouth sub-terminal; pharynx well developed; intestinal crura lacking lateral diverticula. Genital pore ventral. Gonads intercecal. Testes numerous, preovarian; vas deferens loops left intestinal crus; prostatic complex, cirrus, accessory piece present. Ovary compact, single; vagina present; 3 reservoir-like bodies empty into female duct near level of ootype. Vitellaria present; preovarian vitelline commissure connecting with female system. Parasites of freshwater fishes.

***Linguadactyloides* gen. n.**

DIAGNOSIS: Dactylogyridae; Linguadactyloidinae. Body divisible into cephalic region, trunk, peduncle, and haptor. Tegument thin, smooth. Head organs well developed, contained within cephalic lobes and adjacent cephalic area. Cephalic glands in 2 bilateral groups posterolateral to pharynx. Pharynx comprising 2 tandem bulbs; esophagus short or absent; crura confluent posteriorly. Gonads tandem; testes consisting of 3 or 4 bilateral pairs located on each side of midline immediately anterior to ovary. Vas efferentia empty into dilated proximal portion of vas deferens. Oviduct extending along midline ventral to proximal portion of vas deferens; vagina dextral; seminal receptacle a simple dilation of vaginal duct; vitellaria fimbriated laterally. Peduncle with 2 large glandular structures; haptor armed with 2 pairs of anchors, 14 hooks, and ventral bar.

***Linguadactyloides brinkmanni* sp. n.**

(Figs. 1–11)

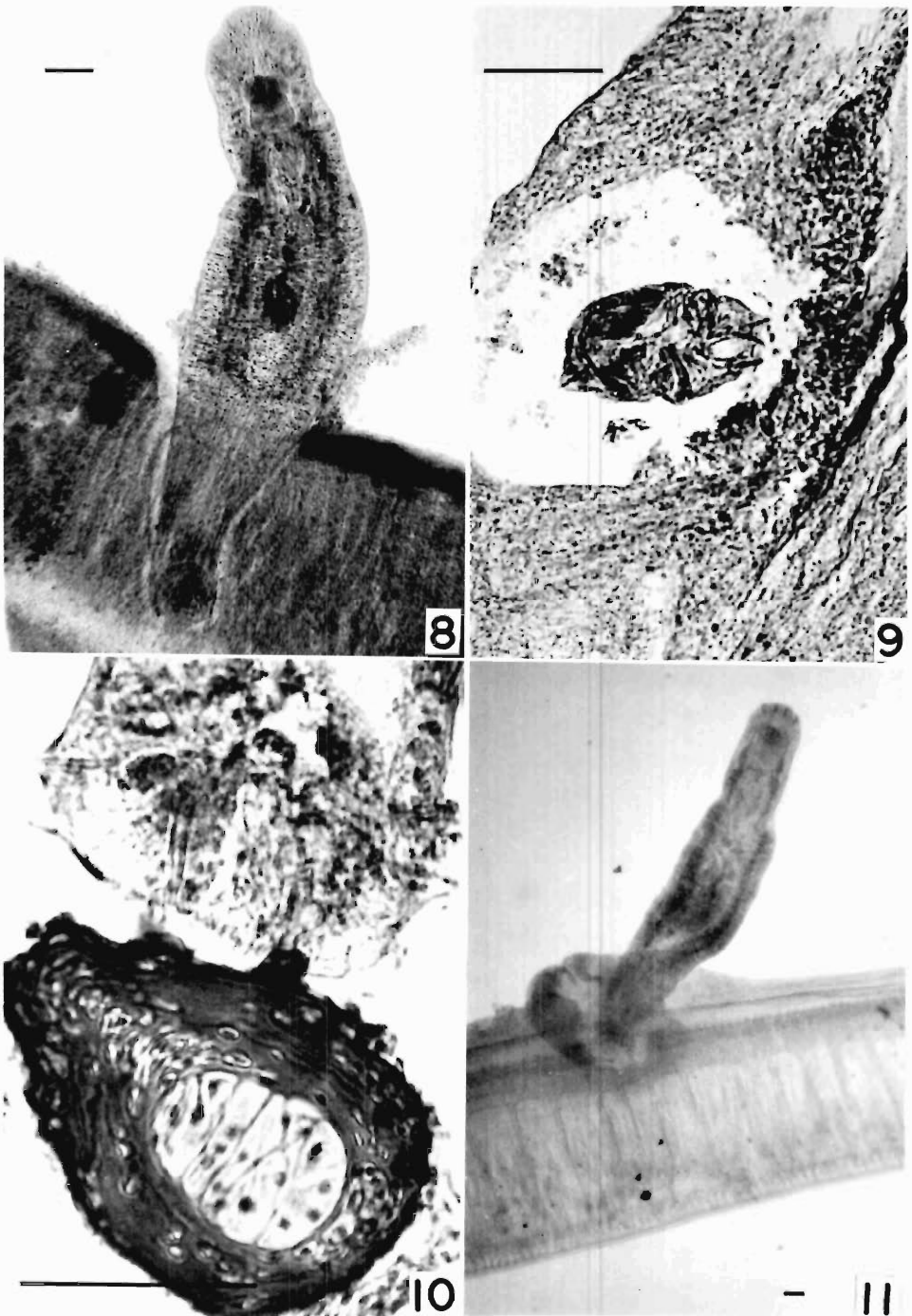
HOST: *Colossoma macropomum* (Cuvier), "Tambaquí," Characidae.

LOCATION: Gills.

LOCALITY: Janauacá Lake, near Manaus, Amazonas, Brazil.

TYPE SPECIMENS: Inst. Nac. Pesq. Amaz. (holotype, paratypes) Nos. PA-233-1, PA-233-2-9; Univ. Nebr. St. Mus., Lincoln (paratype) No. 21478; USNM Helm. Coll., Beltsville, Maryland 20705 (paratypes) No. 77376.

DESCRIPTION (based on 23 specimens): Body fusiform, 1,908 (1,225–3,625) long; greatest width 381 (300–460) near level of testes. Cephalic lobes incipient, 2 terminal, 2 bilateral; each head organ comprising several groups of cephalic gland ducts. Each group of cephalic glands composed of about 8 unicellular glands. Four eyes; members of anterior pair smaller, closer together; component granules small, irregular; accessory granules absent. Posterior pharyngeal bulb usually smaller; greatest pharyngeal width 127 (111–150). Peduncle elongate; haptor 170 (150–207) wide, 165 (142–223) long, a simple extension of peduncle. Hook distribution ancyrocephaline (Mizelle, 1936). Hooks similar; each with erect thumb, attenuated and slightly curved shaft and point, inflated shank; FH loop not observed. Hook prs. 1, 2, 3, 4, 6, 7 are 33 (29–35) long; hook pr. 5 is 25 (23–28) long. Anchors similar; each with straight point, short shaft, perpendicular superficial root, elongate deep root; ventral (anterior) anchor 47 (44–49) long, base 23 (22–24) wide; dorsal (posterior) anchor 44 (42–45) long, base 22 (20–23) wide. Ventral bar 20



Figures 8-11. *Linguadactyloides brinkmanni* gen. et sp. n. 8. Whole preparation showing early penetration of gill and concentrations of blood (dark areas). 9. Cross section of worm in situ, showing haptor in open tunnel and lymphocytic infiltration in tunnel wall (inflammatory reaction). 10. Section showing anchors of haptor engaged in cartilage support of gill filament (with cartilage overgrowing

(15–23) long, broadly “V” shaped, with short medial posterior projection. Testes usually in 4 bilateral pairs; each pyriform, 80 (60–100) by 72 (60–82). Seminal vesicle thick-walled; prostatic reservoirs 2, elongate, thick-walled, parallel, empty individually into base of cirrus by small duct. Cirrus a coil of about 1½ rings; largest ring diameter 101 (94–114). Accessory piece variable, articulated to cirrus base, with terminal sclerotized cirrus guide. Ovary irregular to pyriform, 224 (120–400) long, 127 (80–176) wide. Oviduct large; uterus delicate, directed anteriorly along midline. Vagina with terminal funnel, variably coiled proximal tube; seminal receptacle large, irregular. Vitellaria coextensive with crura; each lateral band with fimbriations dorsal and ventral to intestinal crura.

Discussion

Linguadactyloides brinkmanni gen. et sp. n. is the type species for the genus and is named for Dr. A. Brinkmann, University of Bergen, Bergen, Norway, in recognition of his important contributions to the knowledge of monogenoidean taxonomy. The new species appears to be related to *Linguadactyla molvae* Brinkmann, 1940, which was described from the marine fish *Molva dipterygia* Smitt from Norwegian waters. Features common to both species include the presence of multiple testes, three reservoir-like bodies of the female duct near the ootype, and a single haptor bar supporting the ventral pair of anchors. However, it is differences in these same characteristics that are used to justify the proposal of *Linguadactyloides* gen. n. and Linguadactyloidea subfam. n.

Basic morphological and possibly functional differences in the three reservoirs of the female duct are evident in the two species. Bychowsky (1957) reported that these structures in *L. molvae* are associated with the vitelline duct, into which each opens by a cluster of anterior ducts. He also suggested that they may functionally replace the genito-intestinal canal of higher monogenoideans and serve in the reabsorption of excess spermatozoa, because they usually are filled with mature sperm. In *L. brinkmanni*, however, the reservoirs represent well-defined large vesicles, each of which empties near the ootype by an individual duct. Their contents are granular and do not include spermatozoa, which suggests that they function as reservoirs for glands not seen in our specimens.

Both Brinkmann (1940) and Bychowsky (1957) described the male gonad as being follicular in *L. molvae*, and Yamaguti (1963) gave multiple testes as a diagnostic characteristic of the Linguadactylinae. Our examination of immature specimens of *L. molvae* from Brinkmann's type series (kindly provided by Miss J. Kjennerud, Zoological Museum, Bergen, Norway) supports Yamaguti's interpretation, as they show that development of the “follicles” occurs on an individual basis rather than as outgrowths of a single testis. Also, *L. molvae* possesses vas efferentia apparently arising from each testis, which unite with the vas deferens extending along the ventral surface of the testicular mass (specimen B.M. 41861b, Zoological Museum, Bergen).

The positions of the testes in *Linguadactyla molvae* and *Linguadactyloides*

←

points of anchors). 11. Whole preparation of worm attached to gill filament, showing epithelial hyperplasia. All scales are equivalent to 100 micrometers.

brinkmanni principally serve to separate the two genera and respective subfamilies. In *Linguadactyloides* (Linguadactyloidea) the relatively few testes are prev ovarian, whereas the numerous testes of *Linguadactyla* (Linguadactylinae) occur as an intercecal mass posterior to the ovary. In most Dactylogyridae, to which both of these species belong, the testes or testis are postovarian.

Although the presence of a single haptor bar is not a unique characteristic of these genera (it is a feature of some other species of Dactylogyridae), the fact that all known species in the Linguadactylinae and Linguadactyloidea possess only a single ventral bar lends support to their supposed close relationship. *Mono cleithrium* (Ancyrocephalinae) is the only other dactylogyrid genus from the Neotropical Region characterized by one bar (Price and McMahon, 1966).

Brinkmann (1940) and Bychowsky (1957) stated that adults of *L. molvae* lack eyes. Our examination of the type series of this species has verified that all adults possess an inconspicuous pair of eyes immediately anterior to the pharynx (present in B.M. 41861b, the specimen considered an adult by Brinkmann, 1952). However, the eye granules are minute and easily overlooked. *Linguadactyloides brinkmanni*, on the other hand, has two pairs of conspicuous eyes anterior to the pharynx.

Only three species of Dactylogyridae sensu Yamaguti (1963) are known to possess multiple testes, i.e., *Linguadactyla molvae* Brinkmann, 1940, *Linguadactyloides brinkmanni* sp. n., and *Hareocephalus thaisae* Young, 1968. Each of these species belongs to a separate subfamily as its only included representative. Recently, the Ancyrocephalinae (previously of the Dactylogyridae) was elevated to family status to include, in part, the Linguadactylinae and Hareocephalinae by Bychowsky and Nagibina (1978). We are not convinced that species of the ancyrocephaline group warrant separate family status, and thus consider the Linguadactyloidea and the two previously mentioned taxa as subfamilies of the Dactylogyridae sensu Yamaguti (1963). In either case, however, we do not feel that the Linguadactylinae, Linguadactyloidea, or the Hareocephalinae should be classified with species comprising the Ancyrocephalinae or Ancyrocephalidae, but rather that they represent distinct taxa at the subfamily or family level, depending on whether the classification system of Yamaguti or that of Bychowsky and Nagibina is used, respectively. Discovery of new forms related to *Linguadactyla* or *Linguadactyloides* may indicate that these genera comprise a natural taxonomic grouping, in which case they could form a new family in either system of classification.

Behavior and Pathology

The mode of attachment of *Linguadactyloides* to the host gill differs considerably from that of other dactylogyrid monogenoideans. Typically, these ectoparasites attach to the gill epithelium by means of the anchors and hooks of the haptor and may change location at will. In some genera, including *Linguadactyla*, host epithelium overgrows the haptor, causing the worms to become fixed in one location (Bychowsky, 1957). In the case of *L. brinkmanni*, however, there is active penetration of host tissue by the haptor (Fig. 8). When the posterior end of the worm reaches the cartilaginous supporting rod of the gill filament, the anchors of the haptor directly engage this structure. With time, the cartilaginous tissue over-

grows the points of the anchors, effectively fixing the worm to a permanent location (Fig. 10).

The initial host response to the penetration of *L. brinkmanni* is basically an inflammatory reaction. Both erythrocytes and leucocytes are numerous around the site of the lesion (Figs. 8, 9). A funnel-like tube from the surface to the cartilage support is maintained open by the constant movements of the parasite (Fig. 9). Apparently this lesion hemorrhages, at least initially. Epithelial hyperplasia occurs for some length along the gill filament, and becomes more pronounced at the site of the attached worm. Epithelial overgrowth at this site is probably a continuous process, which is partially checked by the feeding activity of the worm (Fig. 11).

Until host reaction traps the worms in a fixed location, they can, and apparently do, change location from time to time. Living worms on detached gill filaments were seen to move from one gill filament to another. Also, it was found that immersion of fish or gill filaments, for 10–20 min, in a 1:4,000 formalin–water solution, freed some, but not all, of the worms. Thus, the use of this method for treating live fish would probably be only partially effective against this species, reducing the numbers of worms without eliminating the infestation.

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A Revision of North American Species of the Genus *Parametorchis* Skrjabin, 1913 (Trematoda: Opisthorchiidae)

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ABSTRACT: Three species of *Parametorchis* are considered valid and are redescribed. *Parametorchis noveboracensis* and *P. manitobensis* are considered synonyms of *P. complexus*. *Parametorchis canadensis* from mink (*Mustela vison*) and *P. intermedius* from the silver fox (*Vulpes fulva*) are distinguished from *P. complexus* by their smaller size and greater sucker width and oral sucker-pharyngeal width ratios in *P. canadensis*.

Five species of *Parametorchis* Skrjabin, 1913 have been reported from the gallbladders of carnivores in North America: *P. complexus* (Stiles and Hassall, 1894) and *P. noveboracensis* Hung, 1926 from the domestic cat; *P. canadensis* Price, 1929 from mink (*Mustela vison*); *P. intermedius* Price, 1929 from the silver fox (*Vulpes fulva*); and *P. manitobensis* Allen and Wardle, 1934 from sled dogs. Little information other than brief original descriptions of the five species has resulted in considerable differences of opinion as to their generic position. The present paper provides information bearing on that matter from a new collection and reexamination of museum specimens.

Materials and Methods

In 1975, eight flukes identified as *Parametorchis manitobensis* were recovered from the gallbladder of a Siamese cat submitted to the Veterinary Services Division, Alberta Agriculture, Edmonton. They were fixed and stored in 10% buffered formalin until September 1981, when they were washed in distilled water and prepared as whole mounts after staining with Semichon's acetic-carmin. Additional specimens were borrowed from the USNM Helminthological Collection, Beltsville, Maryland. Drawings were made with the aid of a camera lucida and microprojector. Measurements are in micrometers unless otherwise stated, and are given as means followed by the range in parentheses.

Results

Parametorchis Skrjabin, 1913

syn. *Allometorchis* Baer, 1943.

GENERIC DIAGNOSIS (after Yamaguti, 1971): Body flattened, attenuated anteriorly, rounded posteriorly, spined. Oral sucker well developed. Acetabulum nearly as large as oral sucker, in anterior $\frac{1}{2}$ of body. Pharynx large; esophagus short. Ceca terminating in posterior extremity of body. Testes tandem to slightly oblique, lobed or entire, in posterior $\frac{1}{2}$ of body. Ovary lobed or entire. Seminal receptacle lateral or posterior to ovary. Uterus anterior of ovary, surrounding acetabulum and overreaching ceca laterally. Vitellaria forming lateral fields in anterior half of body. Excretory vesicle Y-shaped, stem passing between testes.

Parametorchis complexus* (Stiles and Hassall)*(Fig. 1)**

Parametorchis complexus (Stiles and Hassall, 1894) Skrjabin, 1913:377.

syn. *Distoma (Dicrocoelium) complexum* Stiles and Hassall, 1894:425.

syn. *Parametorchis noveboracensis* Hung, 1926:1.

syn. *Parametorchis manitobensis* Allan and Wardle, 1934:406.

REDESCRIPTION: Body 5.17 (4.16–6.09) mm long by 1.86 (0.96–2.33) mm wide. Oral sucker terminal, 244 (198–292) long by 308 (257–369) wide. Acetabulum sessile, 302 (251–332) long by 315 (257–362) wide; its center 1.29 (1.09–1.65) mm from anterior end of body. Sucker-width ratio 1.00:1.09 (1.00:0.82 to 1.00:1.18). Pharynx 269 (204–292) long by 238 (169–350) wide. Prepharynx absent. Oral sucker–pharyngeal width ratio 1.00:0.73 (1.00:0.58 to 1.00:0.83). Anterior testis 587 (268–780) long by 642 (432–746) wide; posterior testis 706 (438–930) long by 755 (467–885) wide; posterior testis 1.07 (0.55–1.34) mm from posterior end of body. Ovary smooth or slightly lobed, pretesticular, 251 (169–403) long by 355 (204–525) wide. Genital pore lateral to, or slightly anterior to, acetabulum. Vitellaria follicular, in lateral fields from ovary to midway between acetabulum and pharynx, confluent anteriorly or not. Eggs operculate, 28 (26–29) long by 15 (13–16) wide.

HOST: Domestic cat (*Felis catus*), domestic dog (*Canis familiaris*).

SITE OF INFECTION: Gallbladder and bile ducts.

SPECIMENS EXAMINED: *Parametorchis manitobensis*: 8 specimens, Alberta, Edmonton, ex *Felis catus*, authors' coll. (4 specimens deposited USNM Helm. Coll. No. 77419); 9 paratypes, USNM Helm. Coll. No. 8764. *Parametorchis complexus*: 2 co-types, USNM Helm. Coll. No. 8. *Parametorchis noveboracensis*: 1 holotype, USNM Helm. Coll. No. 26628; 1 paratype, USNM Helm. Coll. No. 26629.

Parametorchis intermedius* Price*(Fig. 2)**

Parametorchis intermedius Price, 1929:2.

REDESCRIPTION: Body 2.82 (2.60–3.04) mm long by 780 (770–790) wide. Oral sucker terminal, 164 (146–181) long by 203 (192–213) wide. Acetabulum sessile, 171 (167–175) long by 195 (193–197) wide; 839 (819–858) from anterior end of body. Sucker-width ratio 1.00:0.97 (1.00:0.93 to 1.00:1.01). Pharynx 156 (145–167) long by 139 (131–147) wide. Prepharynx absent. Oral sucker–pharyngeal width ratio 1.00:0.69 (1.00:0.68 to 1.00:0.69). Anterior testis 265 (262–268) long by 242 (239–245) wide; posterior testis 292 (268–315) long by 269 (251–286) wide; posterior testis 511 (499–572) from posterior end of body. Ovary lobed, pretesticular, 118 (101–134) long by 198 (180–216) wide. Genital pore lateral and slightly anterior to acetabulum. Vitellaria follicular, in lateral fields from ovary to midway between acetabulum and pharynx. Eggs operculate, 28 (27–29) long by 15 (14–16) wide.

HOST: *Vulpes fulva*.

SITE OF INFECTION: Gallbladder.

SPECIMENS EXAMINED: 2 paratypes, USNM Helm. Coll. No. 28179.

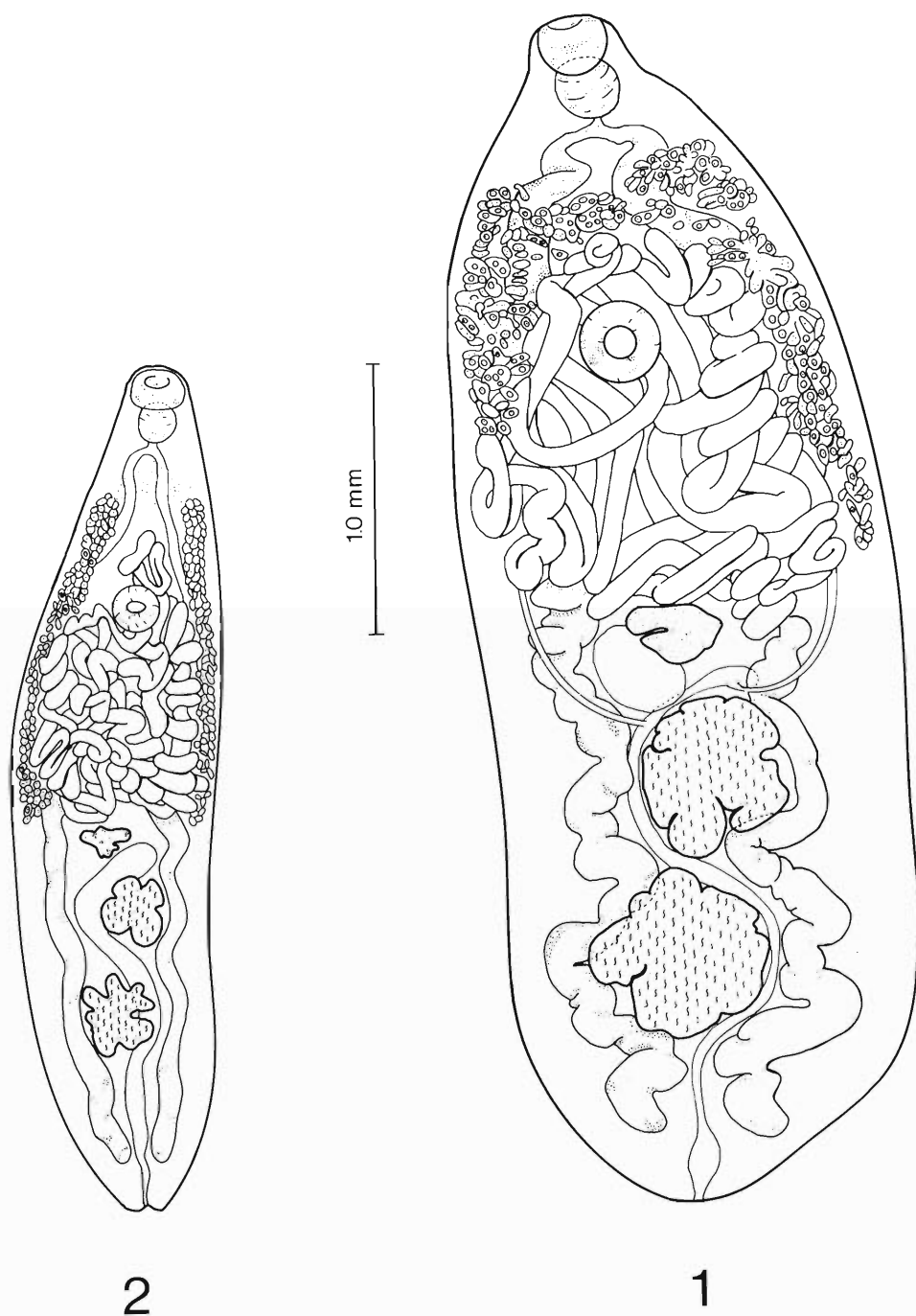
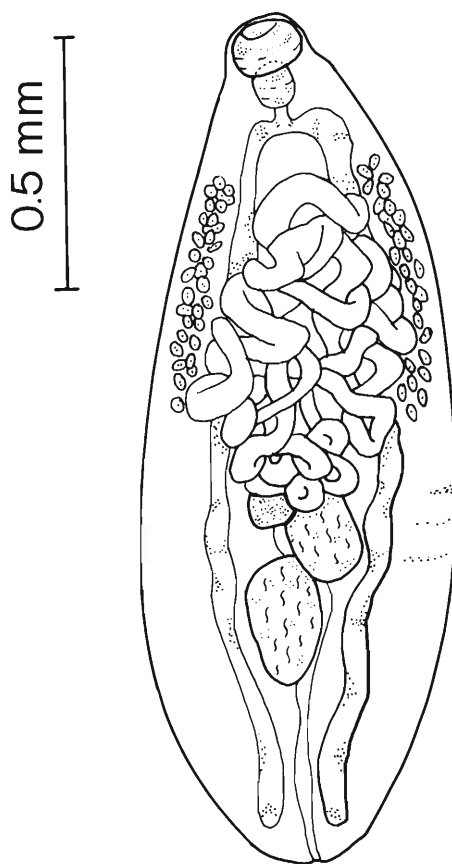


Figure 1. *Parametorchis complexus* (Stiles and Hassall, 1894). Redrawn from cotype (USNM Helm. Coll. No. 8).

Figure 2. *Parametorchis intermedius* Price, 1929. Redrawn from paratype (USNM Helm. Coll. No. 28179).



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Figure 3. *Parametorchis canadensis* Price, 1929. Redrawn from paratype (USNM Helm. Coll. No. 28366).

Parametorchis canadensis Price
(Fig. 3)

Parametorchis canadensis Price, 1929:3.

REDESCRIPTION: Body 1.96 (1.62–2.46) mm long by 580 (550–600) wide. Oral sucker terminal, 100 (89–111) long by 131 (117–145) wide. Acetabulum sessile, 75 long by 73 wide; 367 from anterior end of body. Sucker-width ratio 1.00:0.50. Pharynx 146 (132–160) long by 76 (73–79) wide. Prepharynx absent. Oral sucker–pharyngeal width ratio 1.00:0.59 (1.00:0.55 to 1.00:0.62). Anterior testis 166 (160–171) long by 124 (121–127) wide; posterior testis 192 long by 93 wide; posterior testis 397 from posterior end of body. Ovary lobed, pretesticular, 192 long by 117 wide. Vitellaria follicular, forming lateral bands ranging from anterior level

of ovary to midway between acetabulum and pharynx. Eggs operculate, 28 (27–29) long by 14 (14–15) wide.

HOST: *Mustela vison*.

SITE OF INFECTION: Gallbladder.

SPECIMENS EXAMINED: 3 paratypes, USNM Helm. Coll. No. 28366.

Discussion

Cameron (1940) stated that *P. noveboracensis*, *P. canadensis*, *P. intermedius*, and *P. manitobensis* appear to be synonyms of *Metorchis conjunctus*, but did not provide evidence to support his conclusion. However, the position of the excretory pore does not allow for the inclusion of *Parametorchis* (excretory pore terminal) with *Metorchis* (excretory pore ventral) (Baer, 1943).

Allen and Wardle (1934) used the presence of an anterior band of vitelline follicles to distinguish *P. complexus* from *P. manitobensis*, which does not have a median band. All other measurements of *P. complexus*, based on those provided by Stiles and Hassall (1894) and remeasuring cotypes, are consistent with comparable measurements in *P. manitobensis*. However, only seven of 20 specimens collected by Stiles and Hassall contained median bands. Because of this variation and the variations known to occur in this character for other species of Digenea (Kennedy, 1980a, b), *P. manitobensis* is considered a synonym of *P. complexus*.

Hung (1926) separated *P. noveboracensis* from *P. complexus* by the nearly straight intestinal ceca, unlobed testes, wide pharynx, and large eggs in the former species, and the strongly flexed ceca, lobed testes, and pharynx longer than wide in the latter species. Remeasuring the paratypes and holotype of *P. noveboracensis* showed the pharynx to be longer than wide (320 long by 279 wide) compared to 204 long by 216 wide in *P. complexus*. The differences in shape of the ceca between the two species may be due, in part, to the degree of contraction of the specimens: *P. complexus* (cotype) is markedly contracted, whereas the paratypes and type of *P. noveboracensis* are extended. There are no differences in egg size between *P. complexus* (29 long by 15 wide) and *P. noveboracensis* (29 long by 16 wide). Due to the overlap in all characters measured, and the variations described above, it is impossible to separate *P. complexus*, *P. manitobensis*, and *P. noveboracensis* from one another. The latter two species are here considered synonyms of *P. complexus* (Stiles and Hassall, 1894).

Parametorchis canadensis and *P. intermedius*, described by Price (1929), are the smallest members in the genus, being 1.96 mm long by 580 wide and 2.82 mm long by 780 wide, respectively. Their size alone distinguishes them from previously described species in the genus, and from one another. The lower sucker-width ratio (0.50) and oral sucker-pharyngeal width ratio (0.59) and smooth gonads further serve to separate *P. canadensis* from *P. intermedius*, which has a sucker-width ratio of 0.97, an oral sucker-pharyngeal width ratio of 0.69, deeply lobed gonads, and a larger size of all other features measured.

Acknowledgments

I would like to thank Dr. J. Ralph Lichtenfels of the United States Department of Agriculture, Beltsville, Maryland, for lending me type specimens of the five species of *Parametorchis* discussed in this paper.

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***Sarcocystis montanaensis* and *S. microti* sp. n. from the Meadow Vole (*Microtus pennsylvanicus*)**

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ABSTRACT: Two new species of sarcocysts with compartments were found in skeletal muscles of the meadow vole (*Microtus pennsylvanicus*). *Sarcocystis montanaensis* sarcocysts are $459 \times 199 \mu\text{m}$, smooth, thin-walled ($1.2 \mu\text{m}$), and without protrusions on the wall. Its bradyzoites are $11.1 \times 3.3 \mu\text{m}$ and contain micronemes that extend to the posterior end of the bradyzoite. *Sarcocystis microti* sarcocysts are $319 \times 100 \mu\text{m}$, thick-walled ($2.8 \mu\text{m}$), with protrusions with a central core on the wall. The bradyzoites are $15 \times 3 \mu\text{m}$ and the micronemes are restricted to the anterior end. A cat fed muscles infected with *S. montanaensis* and *S. microti* did not shed sporocysts in its feces.

During the course of a survey for the prevalence of *Toxoplasma gondii* in small mammals in Montana, tissues from *Microtus pennsylvanicus* were also examined for *Sarcocystis* infections. The present report describes two new species of *Sarcocystis* from the meadow vole (*M. pennsylvanicus*).

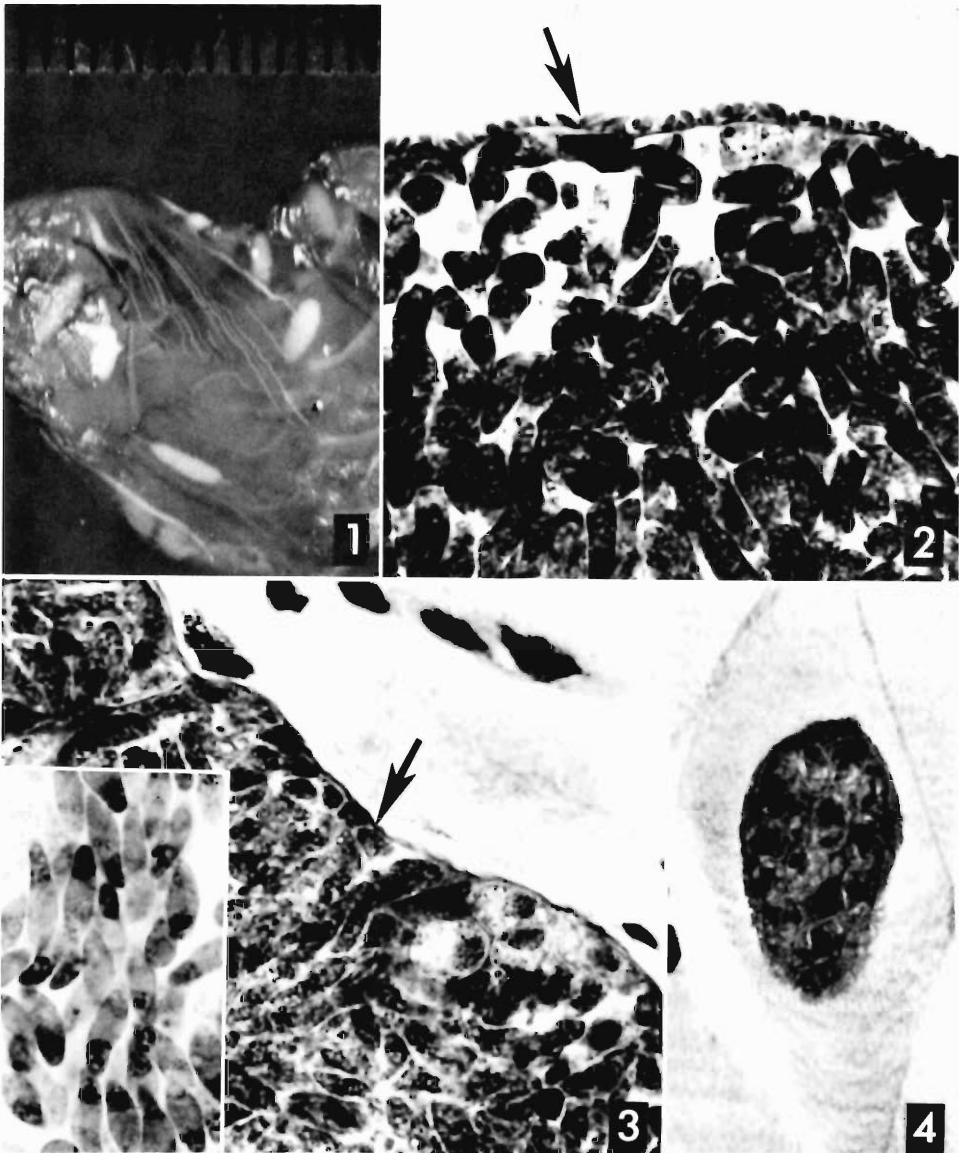
Materials and Methods

Portions of semitendinosus and abdominal muscles, heart, brain, eyes, kidneys, liver, adrenal glands, and lungs of 31 meadow voles (*M. pennsylvanicus*), and eight long-tailed voles (*M. longicaudatus*), trapped around Bozeman from June to December 1980, were fixed in 10% neutral buffered formalin. Paraffin-embedded sections were cut at $5 \mu\text{m}$ and examined after staining with hematoxylin and eosin or periodic acid–Schiff hematoxylin.

Grossly visible sarcocysts in intercostal muscle of meadow vole no. 1 (trapped August 11, 1980) were fixed in 3% glutaraldehyde, processed for transmission electron microscopy 20 mo later, and examined with a JEOL 100 CX electron microscope. Infected muscles from meadow vole no. 1 were fed to a cat. The cat came from the closed cat colony maintained in the Veterinary Research Laboratory, Montana State University, and had never eaten meat before the experiment. Feces of the cat were examined for 30 days for coccidian oocysts, starting from the day of feeding sarcocysts.

Results

Rice grain-like sarcocysts were visible in the intercostal muscles of meadow vole no. 1 (Fig. 1). Histological study revealed sarcocysts in skeletal muscles (Figs. 2, 3). Most sarcocysts were mature and could be easily recognized as two types by the structure of their walls and bradyzoites. Of 47 mature sarcocysts in the semitendinosus muscles, four were thin-walled and 43 were thick-walled; in the same sections nine sarcocysts were immature. Immature sarcocysts were $50.1 \times 18.8 \mu\text{m}$ ($35\text{--}77 \times 14\text{--}25 \mu\text{m}$; $N = 19$) with thin walls ($< 1 \mu\text{m}$), and contained only metrocytes (Fig. 4). The thin-walled sarcocysts are named *S. montanaensis* and thick-walled ones *S. microti*.



Figures 1-4. Sarcocysts in skeletal muscles of *M. pennsylvanicus*. 1. Unstained. 2-4. In semiten-dinosus muscle. Giemsa's stain. $1\ \mu\text{m}$. $\times 1,000$. 1. Macroscopic sarcocysts of *S. montanaensis* in intercostal muscles. Each division of the scale is 1 mm. 2. *S. microti* with villar projections (arrow) on the wall. Bradyzoites have prominent granules. 3. *S. montanaensis* with thin, smooth wall (arrow). Inset shows bradyzoites. 4. Immature sarcocyst.

Sarcocystis montanaensis sp. n.

Under the light microscope, the sarcocysts were $459 \times 199\ \mu\text{m}$ ($270\text{--}648 \times 115\text{--}252$; $N = 4$) and thin-walled ($1\ \mu\text{m}$) (Fig. 3). Six sarcocysts were studied ultrastructurally. They frequently had a very thin rim of host cell cytoplasm between them. The parasitophorous vacuolar membrane (PVM) had minute blebs,

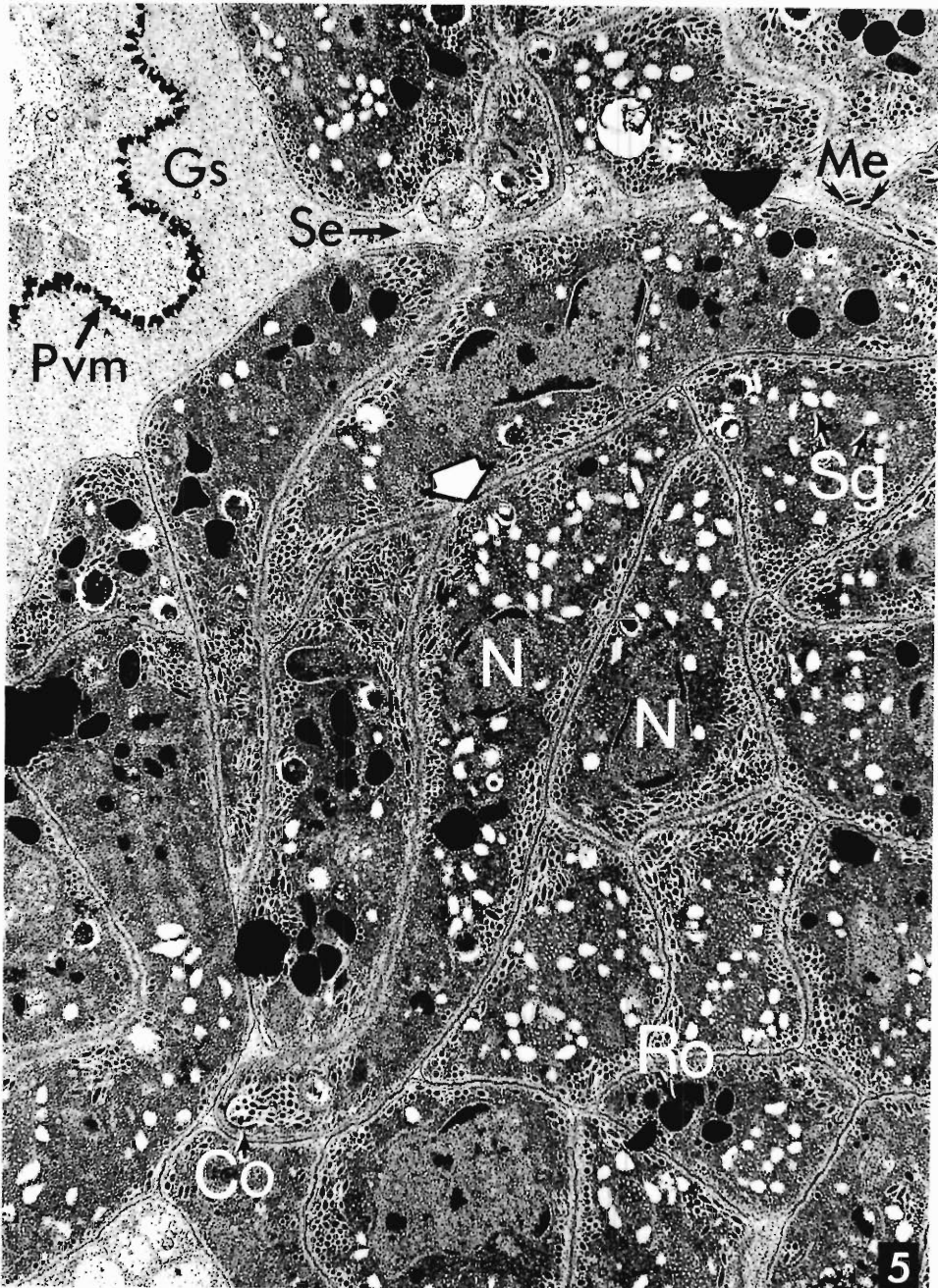


Figure 5. Transmission electron micrograph (TEM) of *S. montanaensis*, showing the parasitophorous vacuolar membrane (PVM), ground substance (Gs) continuing as septum (Se), and numerous bradyzoites. One bradyzoite (large arrow) is cut longitudinally and has a conoid (Co), micronemes (Me), nucleus (N), and rhoptries (Ro). $\times 10,000$.

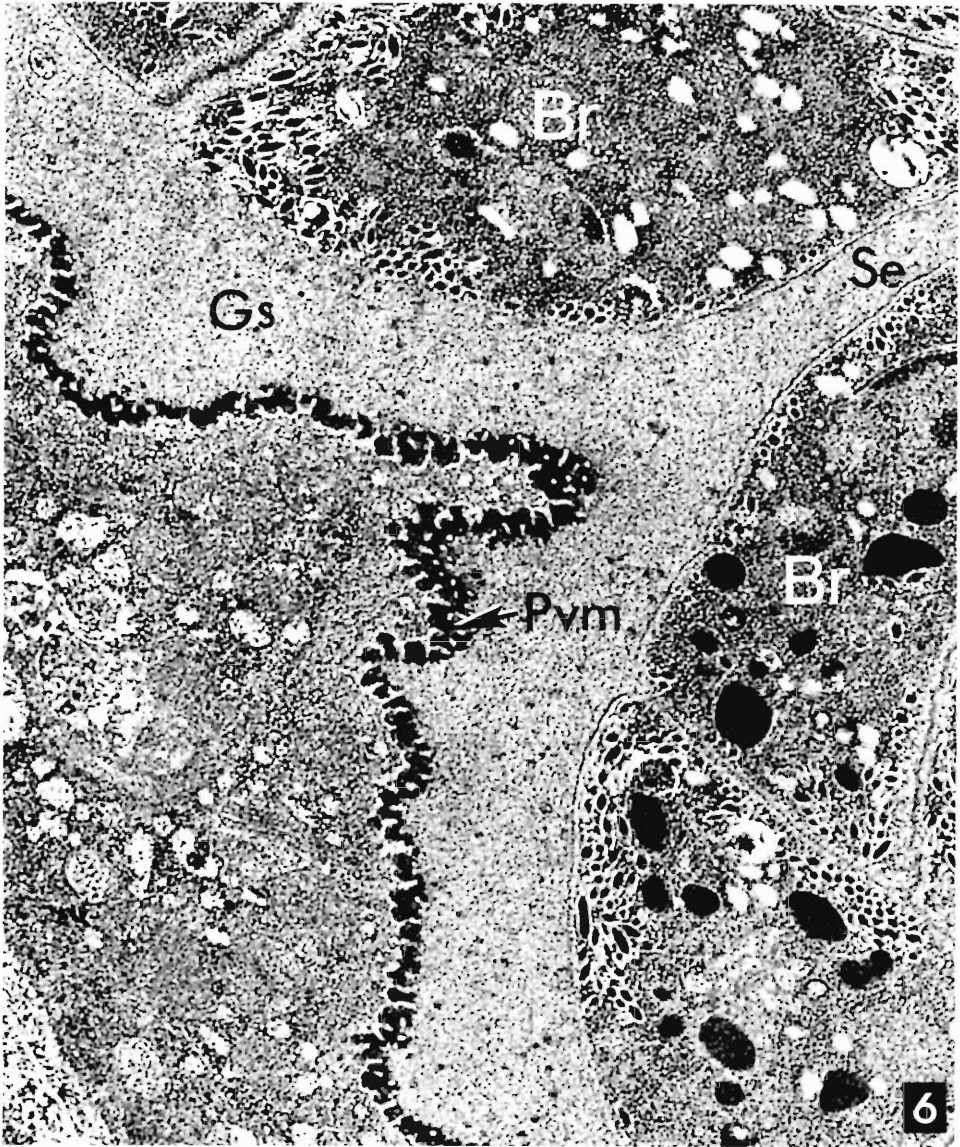
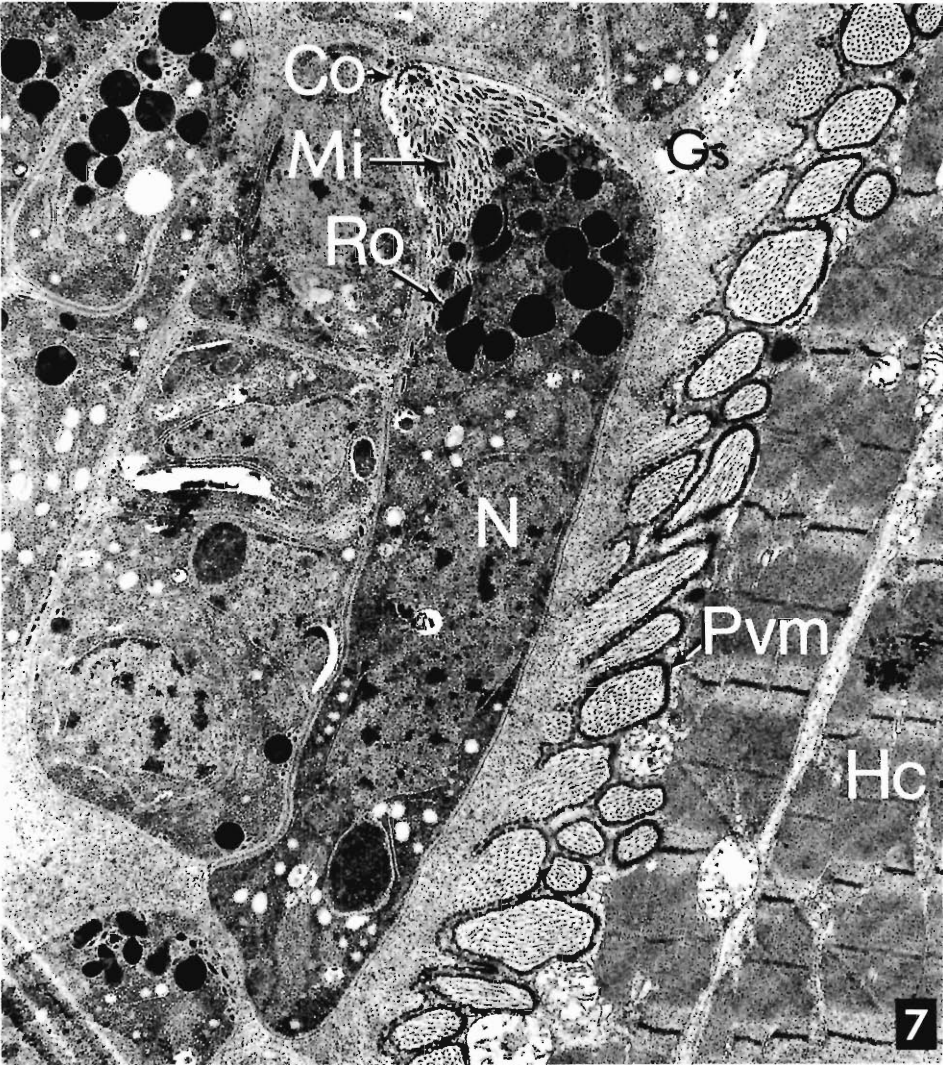


Figure 6. TEM of *S. montanaensis*, showing blebs on the PVM, ground substance, and bradyzoites (Br). $\times 16,600$.

but no protrusions (Figs. 5, 6). Just beneath the PVM was the homogeneous ground substance (Gs), which continued as septa in the sarcocysts. The total thickness of the wall was $1.2\ \mu\text{m}$ ($0.6\text{--}2.1\ \mu\text{m}$; $N = 24$). The septa were $0.6\text{--}1.8\ \mu\text{m}$ thick and divided the bradyzoites into compartments. The bradyzoites were $11.1\ \mu\text{m}$ ($9.8\text{--}12.2\ \mu\text{m}$; $N = 9$) long and $3.3\ \mu\text{m}$ ($2.2\text{--}4.3\ \mu\text{m}$; $N = 27$) wide. They contained numerous micronemes, which extended from the anterior to the posterior end (Fig. 3). Metrocytes were not seen in mature sarcocysts.



Sarcocystis microti sp. n.

Under the light microscope, the sarcocysts were $319 \times 100 \mu\text{m}$ ($108\text{--}1,224 \times 54\text{--}144 \mu\text{m}$; $N = 43$) and had a relatively thick, striated wall (Fig. 2). Four sarcocysts were studied ultrastructurally. The PVM had sloping to straight protrusions (Figs. 7, 8) $2.6 \times 0.56 \mu\text{m}$ ($1.8\text{--}3.3 \times 0.3\text{--}0.9 \mu\text{m}$; $N = 9$), with a central striated core. These striations extended into the ground substance (Fig. 8). The total thickness of the wall (including the ground substance and protrusions) was $2.8 \mu\text{m}$ ($1.7\text{--}4.0 \mu\text{m}$; $N = 11$). The ground substance continued internally as the septa. Bradyzoites were numerous and up to $15 \mu\text{m}$ long and $3.0 \mu\text{m}$ ($2.1\text{--}3.7 \mu\text{m}$; $N = 13$) wide; only one bradyzoite was cut longitudinally. Micronemes were restricted to the anterior ends of the bradyzoites (Fig. 7). Metrocytes were few and were located just beneath the ground substance. They were $7 \times 3.4 \mu\text{m}$ ($6\text{--}8.5 \times 2.5\text{--}5 \mu\text{m}$; $N = 5$). Syntypes (No. 77639) have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705.

Sarcocystis Infection in *M. pennsylvanicus*

In addition to sarcocysts in meadow vole no. 1, one *S. montanaensis* sarcocyst ($252 \times 108 \mu\text{m}$) was found in meadow vole no. 2, and three *S. montanaensis* sarcocysts ($360 \times 90 \mu\text{m}$; $144 \times 36 \mu\text{m}$; $93 \times 47 \mu\text{m}$) were found in meadow vole no. 3. *Sarcocystis microti* sarcocysts ($72\text{--}360 \times 43\text{--}90 \mu\text{m}$; $N = 12$) were found in meadow vole no. 4. An immature sarcocyst ($56 \times 14 \mu\text{m}$) was found in meadow vole no. 5. Thus, five of 31 *M. pennsylvanicus* were infected with *Sarcocystis*; in all instances the sarcocysts were in skeletal muscles.

Sarcocystis Infection in Long-Tailed Voles (*M. longicaudatus*)

Sarcocysts were found in two of eight *M. longicaudatus*. Four thin-walled sarcocysts measuring $73.8 \times 52.2 \mu\text{m}$ ($57\text{--}100 \times 47\text{--}57$) were found in one vole; under the light microscope these appeared similar to *S. montanaensis*. Numerous thick-walled sarcocysts measuring $130.1 \times 62.2 \mu\text{m}$ ($54\text{--}432 \times 36\text{--}90 \mu\text{m}$; $N = 24$) and with striations on the wall were found in another vole; these appeared similar to *S. microti*.

Discussion

Sarcocystis species are generally host-specific, and none was listed by Levine and Tadros (1980) as occurring in the meadow vole. The structure of the sarcocyst wall is a stable criterion for distinguishing *Sarcocystis* species within a given host (Mehlhorn et al., 1976). The structure of the sarcocyst walls from *M. pennsylvanicus* in the present study is different from those of sarcocysts in rats and mice (Mehlhorn et al., 1976; Beaver and Maleckar, 1981). Therefore, the *Sarcocystis* species in the meadow vole were given new species names.

←

Figures 7, 8. TEM of *S. microti*. 7. Periphery of the sarcocyst, showing the protrusions on PVM, ground substance, and longitudinal section of a bradyzoite. The host cell (Hc) is unaltered. The micronemes in the bradyzoite are restricted to the anterior end. A conoid (Co) and rhoptries (Ro) are visible. $\times 10,000$. 8. Higher magnification of protrusions on the wall to show interruptions (arrow) in the PVM and striations in the central core (arrowheads). $\times 32,000$.

Sarcocysts in *M. longicaudatus* appeared similar to those of *S. montanaensis* and *S. microti*; however, without ultrastructural studies their identity could not be established.

Acknowledgments

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The author thanks Harvey Peter Feigley, Gayle Callis, and Merrie Mendenhall for technical assistance.

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Instructions to Authors

Prospective authors may consult “The Journal of Parasitology Instructions to Authors October 1980” (MacInnis, 1980, J. Parasitol. 66(5):865–880) for assistance in preparing manuscripts for the Proceedings. Keep in mind, however, that the Proceedings will consider manuscripts in any and all areas of parasitology.

A few reminders may help to speed the review process: (1) an original and two copies of each manuscript are needed; (2) glossy prints of all halftone illustrations are required for review (xerox copies are unacceptable); (3) reviews of revised manuscripts may be avoided if each point raised by the reviewers and the editor is addressed individually in a covering letter to the editor; (4) the manuscript notation for museum specimens should include the name and address of the museum or collection, and accession numbers (e.g., USNM Helminthological Collection, USDA, Beltsville, Maryland 20705, Nos. 00000, 00000).

EDITORS

Report of Water Mite Larvae in the Esophagus and Stomach Walls of Mountain Whitefish in British Columbia

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ABSTRACT: Larvae of a *Unionicola* species were found embedded in the walls of the esophagus and stomach of *Prosopium williamsoni* (Girard). Twenty-one percent of the 98 mountain whitefish examined were infected, with a mean intensity of 41. The water mite larvae were regarded as accidental parasites originating from chironomid hosts that are part of the diet of the fish.

Reports of water mites in fish have been rare, because they are usually free-living or are parasites of freshwater mussels, sponges, or insects. Larvae of *Unionicola crassipes* (Mueller) have been reported from the walls of the esophagus and pharynx of whitefish *Coregonus lavaretus* in the USSR (Bykhovskaya-Pavlovskaya et al., 1962). In West Virginia, Hoffman (1967) reported unidentified mites encysted in the esophagus of *Lepomis macrochirus* (Rafinesque). Gjernes (pers. comm.) noted *Unionicola* sp. larvae encysted on the swimbladder of rainbow trout, *Salmo gairdneri* Richardson, from Babine and Pennask Lakes in British Columbia in 1969.

The only mites reported for fishes in Canada have been found on the gills (Margolis and Arthur, 1979). This paper contains the first Canadian record of a larval mite found internally. The larvae were identified as a species belonging to the genus *Unionicola* Haldeman, and were found embedded in the muscle layers of the esophagus and stomach walls of mountain whitefish, *Prosopium williamsoni* (Girard). A few larvae of the genera *Hygrobates* Koch and *Piona* Koch were found, but only in the stomach contents. Of 98 mountain whitefish examined from the Kenney Dam area of the Nechako Reservoir in 1979, 21% were infected with the mites, with a range of one to 159 and a mean intensity of 41. Mountain whitefish and lake whitefish, *Coregonus clupeaformis* (Mitchill), were found to be infected with the same larval mite in Stuart Lake in 1981. The prevalence was 79% of 14 mountain whitefish, with a range of one to 18 and a mean intensity of eight mites. One of five lake whitefish was infected with four mites.

Because previous descriptions of water mite larvae from fish are either scarce or inaccurate, it was necessary to attempt to identify the specimens found in the mountain whitefish.

Materials and Methods

The terminology and abbreviations used in describing the water mite larvae are those used by Prasad and Cook (1972). These two authors gave 118 measurements per species; 52 measurements were taken for this study, and were regarded to be a sufficient number for diagnostic purposes. Measurements were taken from five specimens and are given in micrometers.

Sections of the fish esophagus and stomach wall were fixed in Bouin's or 10% buffered formalin. The mites were removed mechanically from preserved tissues. Mites were also removed by digesting fresh stomach tissue using 0.5% pepsin and

Table 1. Lengths (μm) of coxal setae and leg I for four species of *Unionicola*.

Feature	<i>U. gracilipalpis</i>	Prasad and Cook's <i>Unionicola</i> sp.	<i>U. intermedia</i>	Ching and Parker's <i>Unionicola</i> sp.
C1	48–51	62–65	66–70	66–80
C2	50–54	67–75	62–70	82–90
C3	86–93	90–108	84–91	102–112
C4	99–101	115–120	82–88	120–142
Leg I	128–133	138–143	160–168	194–206

5% HCl. The specimens were mounted in Berlese’s chloral-gum solution. Drawings were made with the aid of a camera lucida. A series of specimens has been deposited in the Canadian National Collection (CNC), Biosystematics Research Institute, K. W. Neatby Building, Ottawa.

General Description (Fig. 1)

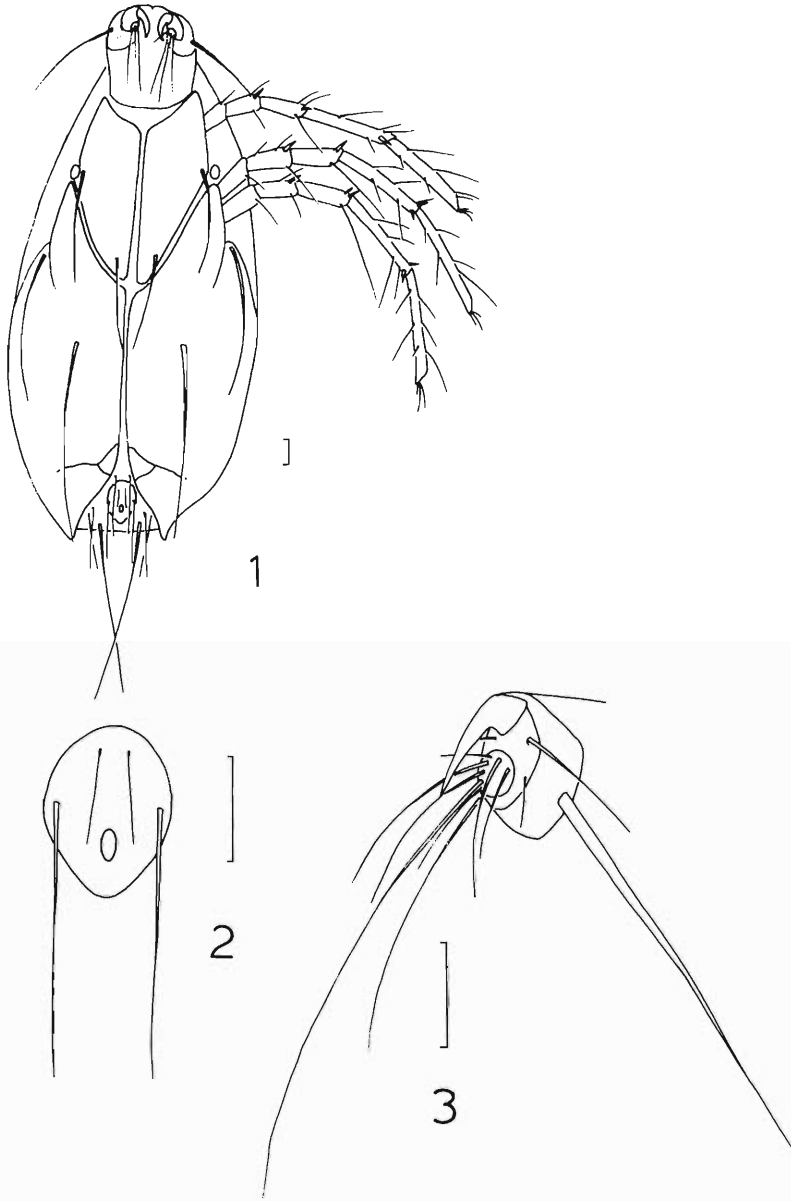
Characteristics of the genus *Unionicola* as shown by the larva are dorsal plate large; 12 pairs of dorsal setae; coxal plate I separate, II and III fused; plates II–III narrowing to a point posteriorly; 4 pairs of coxal plate setae; excretory pore on posterior ½ of excretory pore plate; excretory pore plate with 2 pairs of setae (Fig. 2); 4 pairs of ventral setae V4 very long; cheliceral bases fused; chela edentate; palps very short, tarsus with a long solenidion, some tarsal setae very long (Fig. 3); 3 pairs of legs with 5 segments, each leg with 3 claws, empodial claw bifurcate at tip, eupathidia comparatively long; femur of leg I without long setae; tarsi I and II with 11 setae; solenidia on tibiae II and III long (Figs. 4–6).

Comparisons

Because there are few descriptions and figures of the larvae of *Unionicola* species in the literature, this species was compared to two species for which measurements are given by Prasad and Cook (1972): *U. gracilipalpis* (Viets) and *Unionicola* sp. Prasad and Cook. In addition, measurements were made of larvae of *U. intermedia* (Koenike) from *Anodonta kennerlyi* Lea from Lake Samish, Washington (Table 1). Figures of *U. aculeata* (Koenike) from Mitchell (1955), *U. crassipes* of Bogatova from Bykhovskaya-Pavlovskaya et al. (1962), and the 10 species of *Unionicola* by Hevers (1980) were used for comparisons.

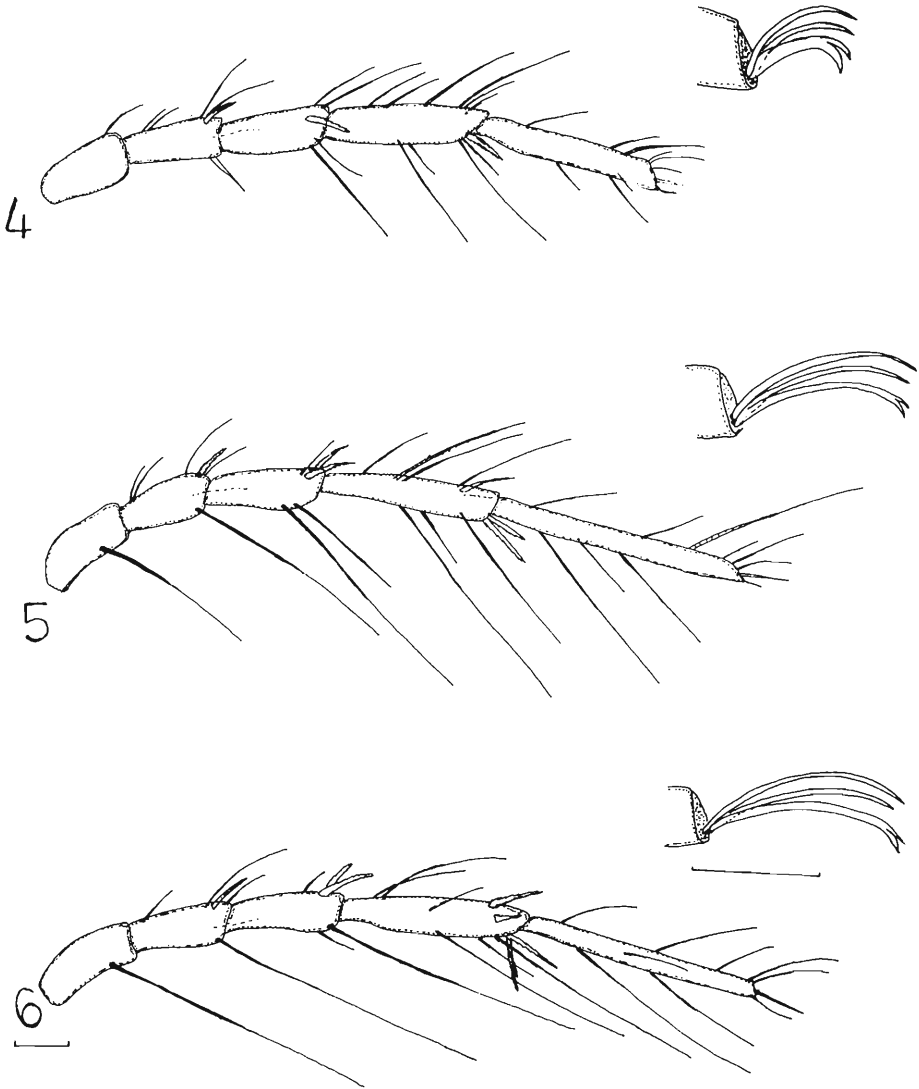
Characteristics of the ventral surface of the idiosoma and the lengths of leg segments were used to distinguish the various species. When coxal setae and leg I measurements of three larvae, *U. intermedia*, *U. gracilipalpis*, and *Unionicola* sp. of Prasad and Cook, were compared they were all less than for the species studied (Table 1). Other differences in measurements of the four species included the lengths of the coxal plates, the lengths of the first pair of excretory pore plate setae, and the distance between this pair of setae.

The excretory pore plate of the larva studied was oval, narrowing posteriorly, with the maximum width at the level of the E2 setae (Fig. 2). The other species differed from the above as follows: *U. intermedia*—round with blunt posterior tip; *Unionicola* sp. of Prasad and Cook—round; *U. gracilipalpis*—elongate, with blunt posterior tip; *U. aculeata*—sharply pointed anteriorly; *U. crassipes* of Bo-



Figures 1-3 *Unionicola* sp. 1. Ventral view of larva showing legs on one side only. 2. Excretory pore plate. 3. Genu, tibia and tarsus of palp. Scale lines = 20 μ m.

gatova—triangular. Comparisons of the 10 species of *Unionicola* figured by Hevers (1980) with the larva studied showed the excretory pore plates to be distinctly different. The excretory pore plate of the large form of *U. crassipes crassipes* was most similar to that of the larva in the size and placement of E1 and E2 setae. However, the maximum width of the excretory pore plate is in the posterior half,



Figures 4-6. *Unionicola* sp. Legs I, II, and III, showing setae with enlargement of claws. Scale lines = 20 μ m.

past the E2 setae. Measurements of C4 and legs I, II, and III were similar, but other measurements were not available for comparison.

In conclusion, available data on *Unionicola* larvae showed characteristics unique to each species. However, the larva studied could not be identified specifically from the available descriptions and figures. It is most similar to that of *U. crassipes*, but cannot be assigned to species until other stages in the life cycle and its natural hosts are known.

Discussion

Böttger (1972) described the life cycle of *Unionicola crassipes* from freshwater sponges and from chironomid larvae, pupae, and adults from a pond in West Germany. One phase of the life cycle involved a phoretic association between the larva of *Unionicola crassipes* and chironomid larvae. *Unionicola* larvae are typically ectoparasitic on the imagoes of chironomids (Smith and Oliver, 1976). The larval stages of the *Unionicola* sp. and of chironomid spp. occurred in the stomach contents of the mountain whitefish. Although chironomid larvae were a common diet item, the *Unionicola* larvae were not digested and burrowed into the muscle layers of the walls of the esophagus and stomach of the fish. No host reaction such as encystment was observed. Whether the larvae were alive within the tissue could not be determined because the fish had been frozen prior to examination. No developmental stages (protonymphs or deutonymphs) other than the larvae were found, which indicated that the development of the mite had been arrested. It was concluded that the ingested water mite larvae were accidental parasites of the fish.

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Research Note

Resistance of Calves to Reinfection with *Oesophagostomum radiatum*

Resistance to infection with the intestinal nematode *Oesophagostomum radiatum* is evoked in calves by intravenous injection with infective larvae L₃ (Herlich, 1971, J. Parasitol. 57:504–507) and intraperitoneal injection with in vitro-grown parasitic third- and fourth-stage larvae (Herlich, Douvres, and Romanowski, 1973, J. Parasitol. 59:987–993). However, single doses of rather massive numbers of larvae were used in those studies, and extensive lesions were produced in the lungs, mesentery, and lymph nodes. Single oral doses of as few as 1,000 L₃ and daily doses of 50 L₃ (total inoculum = 5,400 L₃) stimulated strong resistance to challenge inoculations (Roberts, Elek, and Keith, 1962, Aust. J. Agr. Res. 13:551–573). The present study was designed to determine the minimum number of L₃ that would stimulate resistance.

Thirteen 3-mo-old Holstein steers reared helminth-free were allotted to five groups; three calves were in each of Groups I, II, and V and two calves each in Groups III and IV. The calves were inoculated orally with L₃ as follows: Group I—50/calf; II—500; III—1,000; and IV—5,000. Group V calves served as uninoculated controls. At 18 wk postinoculation (PI) all calves in Groups I and IV were given 7.5 mg/kg levamisole orally to terminate their infections. One week later, each calf in all groups was orally inoculated with 20,000 *O. radiatum* L₃.

The course of initial and challenge infections was monitored by weekly fecal egg counts. Calves were necropsied 52 days after challenge inoculation to determine the numbers of *O. radiatum* present.

The results are summarized and presented in Table 1. During the initial infection the maximum eggs per gram (epg) per group was at Weeks 13 and 14, and there was little or no drop in epg through Week 18, when the infections were chemically terminated. The epg of Group I was much lower than that of Groups II, III, and IV, which did not differ from one another despite the differences in numbers of L₃ administered. There was no evidence of clinical parasitism in any group. Eggs were not detected for 40 days in any calves in Groups I–IV after the anthelmintic was administered. During the postchallenge phase patent infections were established in all calves; epg did not differ markedly among the previously infected and challenge control groups. However, in Group I, the individual calf epg and group average epg were higher than during the initial infection, whereas individual calf maximum epg and average epg were lower in Groups II–IV. On the basis of numbers of *O. radiatum* recovered at necropsy, there was evidence of strong resistance in some calves in all of the immunized groups as compared with the controls, but there were substantial numbers of *O. radiatum* present in one calf of each group. Indeed, Calf 1887 of Group I had the greatest number of *O. radiatum* recovered, over twice the average in the challenge controls. The degree of resistance elicited by preliminary inoculation with 1,000 and 5,000 L₃ (Groups III and IV, respectively) was not as intense as that reported by Roberts et al. (1962, loc. cit.). Those researchers had challenged calves at about 10–14 wk after initial inoculation and infections were still patent. They concluded that calves

Table 1. Results of immunizing and challenge infections with *Oesophagostomum radiatum* in calves.

Group/calf no.	Immunizing dose (no. infective larvae)	EPG (maximum)*		No. worms at necropsy		Percent reduction
		Initial infection	Challenge infection	Total	L ₄	
I/1887	50	56	1,384	8,890	0	0
1889	50	38	64	55	0	98.7
1893	50	42	102	480	5	88.4
Average	—	31	517	3,142	1.7	23.4
II/1883	500	1,028	242	700	50	82.9
1886	500	498	40	285	0	93.0
1897	500	352	308	130	5	96.8
Average	—	480	132	372	18.3	90.9
III/1896	1,000	836	4	70	0	98.3
1898	1,000	784	146	1,230	0	70.0
Average	—	718	75	650	0	84.1
IV/1892	5,000	560	242	1,000	0	75.6
1895	5,000	480	208	460	0	88.8
Average	—	546	225	730	0	82.2
V/1882	None	—	312	5,020	0	
1890	None	—	224	1,740		
1900	None	—	600	5,530	0	
Average	—		352	4,097		

* EPG = eggs per gram of feces. Maximum = single-day maximum count during the prechallenge and postchallenge intervals, respectively.

were resistant, because there was no resultant increase in epg attributable to the challenge. When their cattle were necropsied about 2 wk after a second challenge inoculation, they had none to less than 15 adult *O. radiatum*, but all had fourth-stage larvae, presumably from the challenge inoculum. The difference in the resistance stimulated by a single dose of either 1,000 or 5,000 L₃ in the present study and in the study of Roberts et al. (1962, loc. cit.) may have been due to premunition in the latter study, where infections were patent at time of challenge, or to differences in *O. radiatum* strains and/or cattle (Holstein in Herlich and Shorthorn in Roberts et al. studies). The results of the present study indicate that a single dose of as few as 50 L₃ may stimulate strong resistance in some calves against challenge inoculation. Because 50 L₃ was the smallest inoculum used, there is also the possibility that even fewer L₃ might stimulate some degree of resistance. The results of this experiment emphasize the variability in immune response of calves to helminth infection, even in such small groups as were used.

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Research Note

***Tetrameres grusi* (Nematoda: Tetrameridae) from
Foster-Raised Whooping Crane**

A juvenile whooping crane (*Grus americana*) that had been raised by greater sandhill crane (*Grus canadensis tabida*) foster parents was attacked and killed by a golden eagle (*Aquila chrysaetos*) southwest of Rangely, Colorado (Windingstad et al., 1981, Auk 98:393–394). Since 1975, whooping crane eggs have been placed in the nests of sandhill cranes in an effort to increase the population of this endangered species. This bird was hatched at Grays Lake National Wildlife Refuge in Idaho and was attacked while migrating to wintering grounds in New Mexico. The bird was sent to the National Wildlife Health Laboratory for a detailed necropsy.

A parasitological examination of the gastrointestinal tract revealed a heavy nematode infection in the glands of the proventriculus. The nematodes were subsequently identified as *Tetrameres grusi* Shumakovich, 1946; 86 females were removed from the glands and one male was found in the lumen. No other gastrointestinal parasites were observed. A histological examination showed that *T. grusi* males and females were present in the proventricular glands. A small amount of pressure atrophy was seen associated with gravid females, but there was no inflammatory response.

Tetrameres spp. in other bird species are usually group-specific and light infections generally do not cause damage to the proventriculus. The effect of heavy infections is relatively unknown for most wildlife species.

Although *T. grusi* has been reported from sandhill cranes collected in Florida (Bush et al., 1973, J. Parasitol. 59:788–792), this is the first report of this species in whooping cranes. On one occasion, female *Tetrameres* were removed from the proventriculus of a captive whooping crane during necropsy, but the species identification could not be determined. Representative specimens of *T. grusi* were deposited in the USNM Helminth Collection, Beltsville, Maryland 20705 (No. 77010).

The biological significance of this observation, or lack of it, cannot be determined from this one case, however it illustrates the need for greater understanding of host–parasite relationships, in a broad sense, and suggests the need for additional studies in that area when contemplating wildlife transplants.

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Research Note

Dipetalonema dracunculoides in Dogs and Spotted Hyena
(*Crocuta crocuta*) in the Turkana District of Kenya

During the course of studies on filariasis in Kenya, Nelson found that domestic dogs could be infected with as many as six different species of filarial worms (Nelson, 1963, J. Helminthol. 37:235–240). He examined 27 dogs in the Turkana District of northern Kenya and found 11 to be infected with a single species, *Dipetalonema dracunculoides* (Nelson, 1963, loc. cit.). As part of a study by the African Medical and Research Foundation (AMREF) to determine potential reservoirs of human hydatid disease in Turkana District, an opportunity was made available to examine dogs for hemoparasites as well.

Turkana District occupies the extreme northwestern part of Kenya (Fig. 1). A description of the region and its people was given by Macpherson (1981, Trans. Roy. Soc. Trop. Med. Hyg. 75:680–681). Dogs were collected in three different locations within the District (Table 1, Fig. 1). Each dog was euthanized by an overdose of sodium pentobarbitone (Euthatal®). During necropsy, 2 ml of cardiac blood were drawn. One ml was processed by the concentration technique of Knott (1930, Trans. Roy. Soc. Trop. Med. Hyg. 33:191–197) and the remainder was used to prepare thick and thin blood films. Touch preparations were made of liver and spleen tissue from each dog. Blood films and touch preparations were fixed in methanol and stained with Giemsa. Pieces of liver and spleen were also fixed in 10% buffered formalin and processed for histological examination.

Examination of blood films and sediment from Knott's samples revealed that 50 of the 63 dogs surveyed (79%) were infected with *D. dracunculoides*. The occurrences of infection by location and sex of the dog are listed in Table 1. Knott's samples from two spotted hyenas (*Crocuta crocuta*, one adult ♀ and one juvenile ♂) collected as part of the hydatid survey in the vicinity of Lokomarinyang also contained microfilariae of *D. dracunculoides*. Morphology and dimensions of the microfilariae from both dogs and hyenas correspond to the description given by Nelson (1963, loc. cit.) and are well within the range given by Wolfe and co-workers for *D. dracunculoides* in Pakistan (Wolfe et al., 1971, J. Helminthol. 45:171–176). Microfilariae averaged 255 µm in length by 4.5 µm in width. No

Table 1. The occurrence of *Dipetalonema dracunculoides* in dogs in Turkana District, Kenya.

Location	Sex of dog		Total
	Male	Female	
Lokitaung	19/21 (90)*	14/16 (88)	33/37 (89)
Lokori	6/8 (75)	8/15 (53)	14/23 (61)
Lokomarinyang	2/2 (100)	1/1 (100)	3/3 (100)
All locations	27/31 (87)	23/32 (72)	50/63 (79)

* No. of dogs infected/no. of dogs examined (% infected).

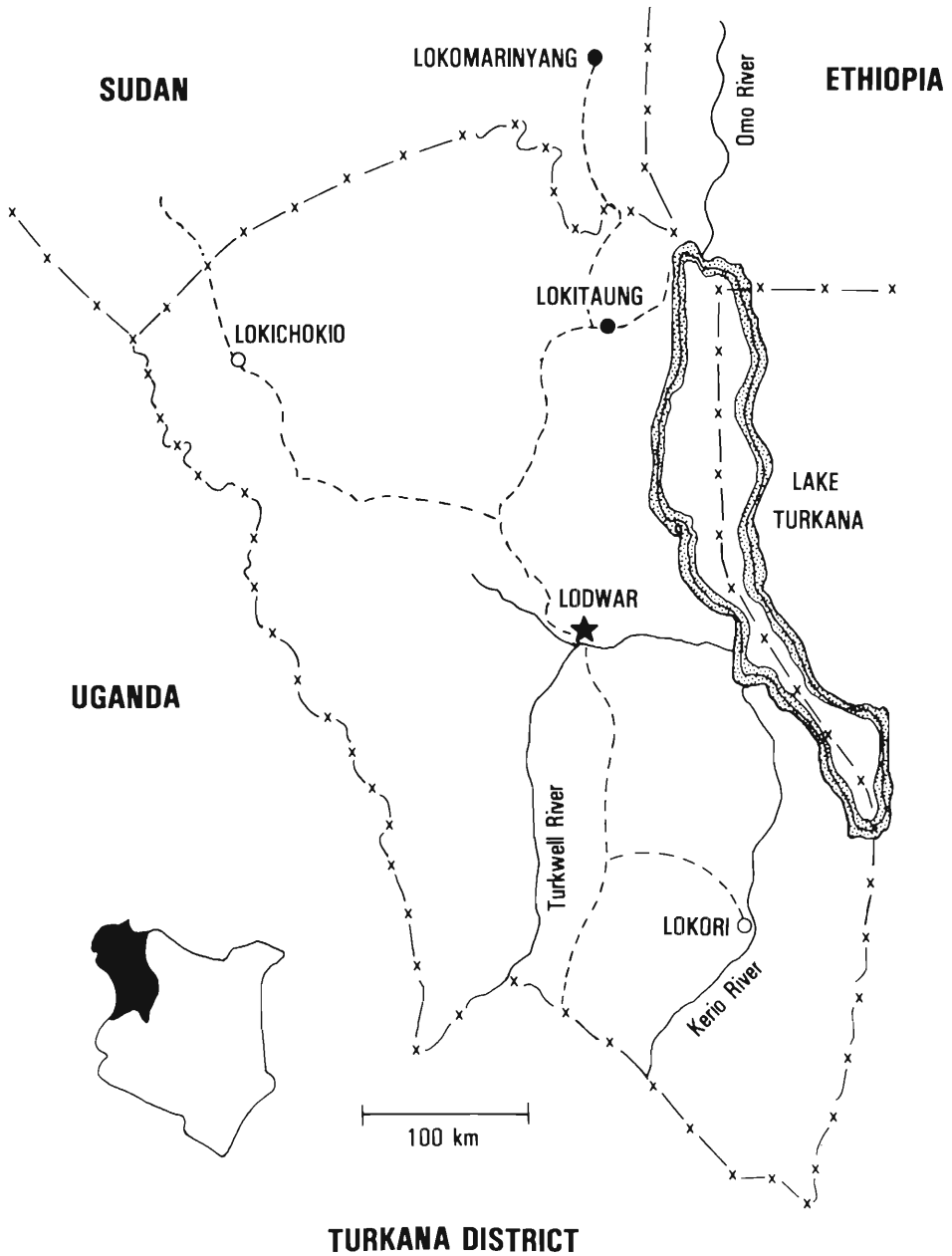


Figure 1. The Turkana District of Kenya, with locations where dogs were examined indicated.

other types of microfilariae were observed in any of the blood samples. Protozoan parasites were also absent in blood samples, however the hemogregarine *Hepatozoon canis* was seen in spleen sections of seven of 36 dogs (19.4%) collected in Lokitaung.

Nelson (1963, loc. cit.) originally described *D. dracunculoides* from dogs and hyenas in Kenya, and maintained that the distribution of the parasite in Africa

coincides with the distribution of the louse fly, *Hippobosca longipennis*. All animals examined in this study were heavily infested with louse flies. Results of this survey indicate that infection of dogs with *D. dracunculoides* is widespread in northern Kenya and that, at present, it is the predominant, if not the only, canine filariid in this region.

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Research Note

Occurrence of *Nematodirella* sp. (Nematoda: Trichostrongyloidea) in Domestic Sheep (*Ovis aries*) of Wyoming

Nematodes of the genus *Nematodirella* Yorke and Maplestone, 1926 were found to inhabit the small intestine of sheep in Wyoming many years ago by Honess (1951, Wyo. Agric. Exp. Stn. Bull. 305:23 pp.). *Nematodirella* species have been reported in sheep in the USSR and other parts of Asia (Skrjabin et al., 1954, Osnovy Nematologii, Vol. 3. Acad. Sci. USSR, Moscow [English version available as OTS60-21124 from NTIS, Springfield, VA 22151]) and in the wild sheep of Iran (Eslami et al., 1979, Iran J. Wildl. Dis. 15(2):263–265). Because it has been seen only once previously, some doubt has persisted as to the presence of the genus in domestic sheep in the United States (Gilmore and Allen, 1960, Proc. Helminthol. Soc. Wash. 27(1):69–72).

Intestinal tracts from 35 lambs and adult sheep from the University of Wyoming herd were used in gathering these data. All sheep were taken from pasture. For sheep Numbers 1 through 9, serial lengths (5–15 ft) of the small intestine were stripped of contents, washed, and the contents screened and examined for the presence of the nematodes. No *Nematodirella* were found in the cecae of two lambs that carried *Nematodirella* in the caudal one-third of the small intestine. Only the caudal one-third to one-half of the intestine was examined in sheep Numbers 10 through 35.

The results of the present study (Table 1) show a range of zero to 398 *Nematodirella*, a mean of 17.5 worms per sheep, and a prevalence of 57%. Honess (1951, loc. cit.) reported a range of zero to 219 worms per sheep, with a prevalence of 15% in 123 sheep examined. The difference in location of these *Nematodirella* sp. in the small intestine, as compared to those in pronghorn antelope and moose, suggests a possible reason for the relatively low burdens noted by Honess and the

Table 1. Numbers and prevalence of *Nematodirella* sp. in lambs and adult sheep in a Wyoming flock.

No. of sheep	Range and mean no. of <i>Nematodirella</i>	Date	Percent positive
3	0–6 (2.7)	Sept. 1980	66
6*	0–398 (70.6)	Oct. 1980	83
7	1–61 (16.8)	Nov. 1980	100
10	0–16 (3)	Jan. 1981	40
9	0–30 (3.6)	Jan. 1982	22
Total 35	612 \bar{x} = 17.48 worms/sheep		\bar{x} = 57

* One sheep also harbored several thousand *Nematodirus* sp.

present authors. We have no explanation for the difference in prevalence in the two studies.

The worms were deep pink to dark red when first recovered from the intestine. All the specimens were recovered from the caudal 25 ft of the small intestine, with a few coming from the last few feet. The anterior portion of the small intestine was inhabited by relatively large numbers of *Nematodirus* sp. in many of the sheep examined, but individuals of that genus did not inhabit the posterior half of the intestine in any significant numbers. Eggs taken from the uterus of the *Nematodirella* specimens and from *Nematodirus spathiger* cultured at room temperature (21°C) at a depth of 5 mm in tap water developed at similar rates. Eggs were in the normal four- to eight-blastomere stage when taken from the female worm uteri. The first larval stage occurred on day 3 and the third larval stage appeared on day 10. Most eggs hatched within a month after the third larval stage was reached.

The present report, following Honess (1951, loc. cit.), is the first in 30 years to verify the occurrence of *Nematodirella* in domestic sheep in the United States. Perhaps the genus is present also in sheep outside the state of Wyoming, but because of relatively low numbers per host animal, it has probably been overlooked. The evidence herewith indicates that various species of the Nematodirinae inhabit specific zones of the small intestine, and therefore the entire tract should be examined in serial sections. Processing the tracts in the serial method mentioned above may aid in the isolation of various species of the Nematodirinae.

Evidence has recently been provided (Lichtenfels and Pilitt, pers. comm.) that the genus *Nematodirella* has evolved in two separate lines, one in cervids and the pronghorn antelope and another in camels and bovids. The specimens from sheep belong to the group of species parasitic in bovids, but additional work is needed to determine the species.

The authors wish to recognize the cooperation of Dr. Melvin Riley and B. Vimini of the Animal Science Department, University of Wyoming, for their assistance in obtaining intestinal tracts, and the advice of Dr. J. Ralph Lichtenfels, Animal Parasitology Institute, Beltsville, Maryland, who confirmed the identification of the *Nematodirella* specimens.

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Research Note

Surface Ultrastructure of the Human Liver Fluke, *Clonorchis sinensis*

The common oriental human liver fluke *Clonorchis sinensis* was first found and reported by McConnell (1875, Lancet ii:271–274). He gave the primary morphological description of the adult worm as well as the eggs. Later, Kobayashi (1917, cited in Faust and Khaw, 1927, Amer. J. Hyg. Monogr. Ser. No. 8:9–117), Chen Pang (1924, cited in Faust and Khaw, 1927, loc. cit.), and Faust and Khaw (1927, loc. cit.) gave in more detail complementary descriptions of the morphology of adult worms and eggs. Observations on the morphology of metacercariae, cysts, and excysted larvae have been reported by many authors since the beginning of this century. Hsü and Khaw (1936, Chin. Med. J. 50:1609–1620) redescribed them in detail. Komiya and Tajimi (1941, cited in Komiya, 1965, Prog. Med. Parasitol. Jpn. 2:1–309) gave additional data. All the above results were observed by using the light microscope. Recently, Ishii and Tokunaga (1971, Chin. J. Microbiol. 4:123–131) reported on the morphology of *C. sinensis* eggs using the scanning electron microscope (SEM). This paper presents further morphological observations on metacercariae, adult worms, and eggs based on the use of SEM.

Metacercariae of *C. sinensis* were obtained from naturally infected fish, *Pseudorasbora parva*, from a suburb near Beijing, China. Adult worms were obtained from experimentally infected cat bile ducts and gallbladders. Eggs were obtained from the uteri of adult worms. All the above materials were treated and prepared for SEM observations based on techniques described by Voge et al. (1978, J. Parasitol. 64:368–372). A ETEC Autoscan was used for observation.

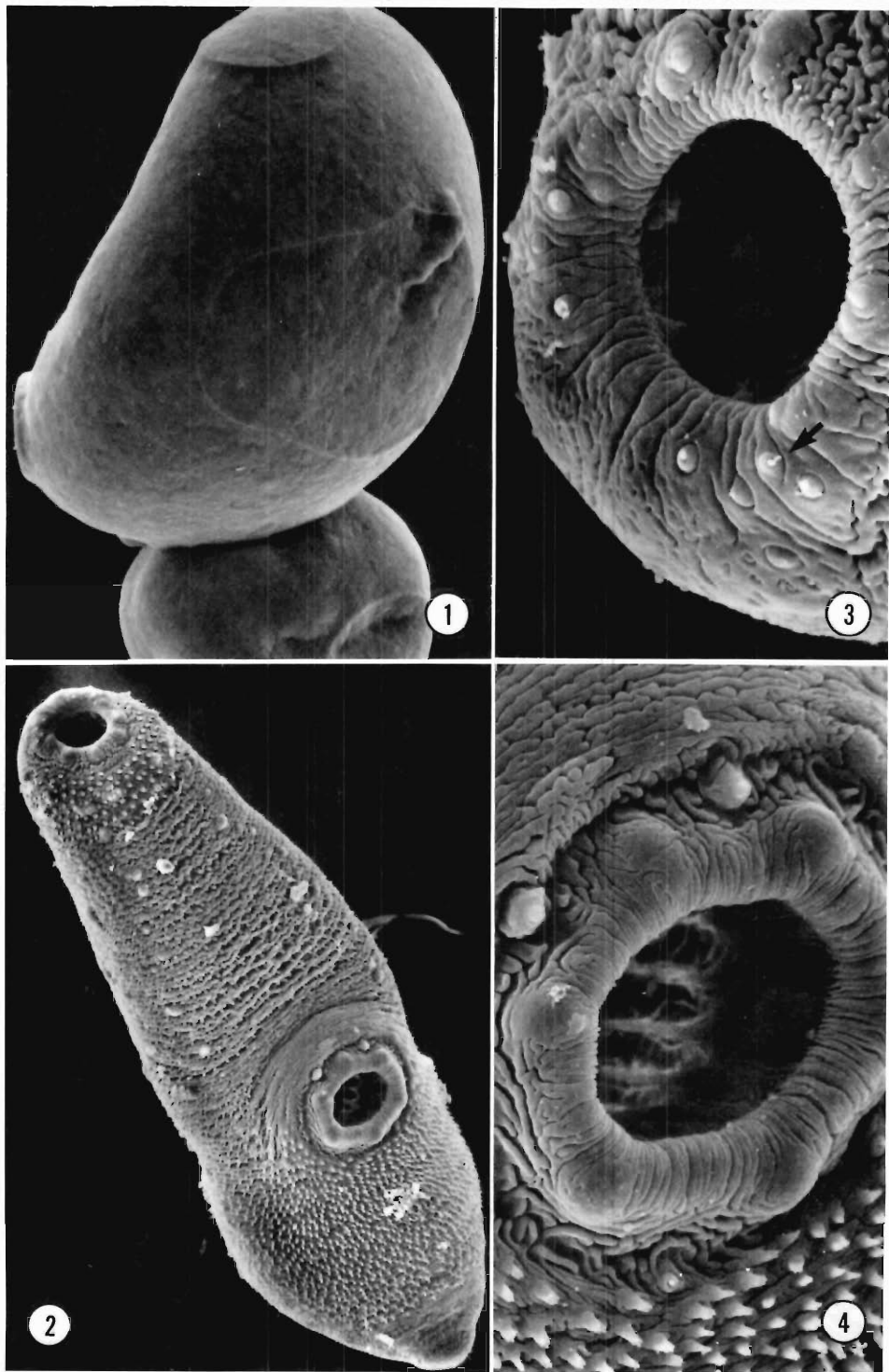
The encysted metacercaria is usually spheroid or ovoid in shape, but may be variable due to movement of the larva inside. The surface of the metacercaria is fairly smooth (Fig. 1). On one side of the surface, a circular outline can be seen, perhaps the site of attachment to the flesh of the fish intermediate host.

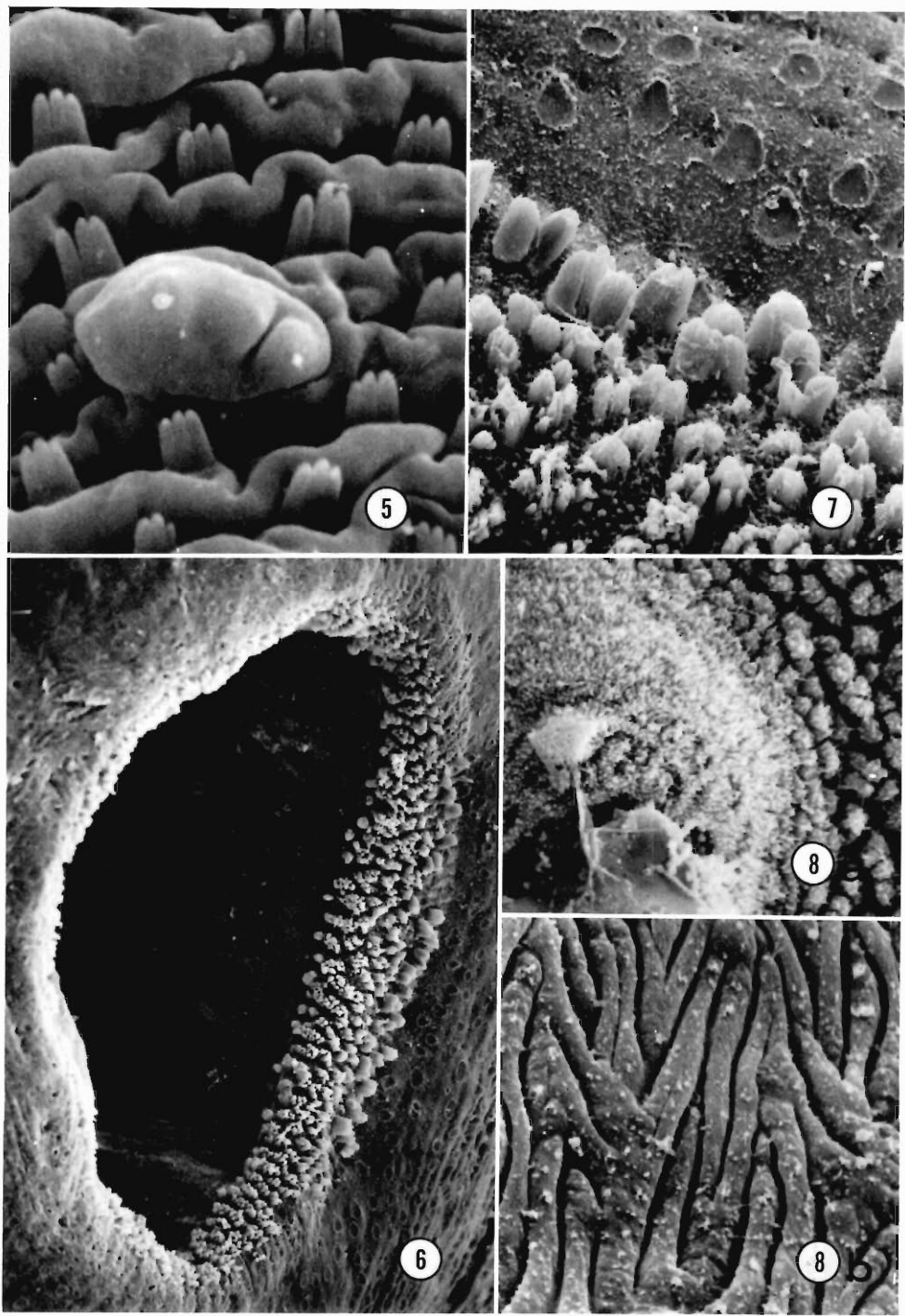
The excysted metacercaria is elongated and ellipsoid in shape (Fig. 2). The oral sucker (Fig. 3) bears eight equidistant protrusions, each with a short knob (cilium?) in the center. These structures may be sensory papillae. The acetabulum (Fig. 4), situated slightly posterior to the middle of the body, also is rounded in shape,

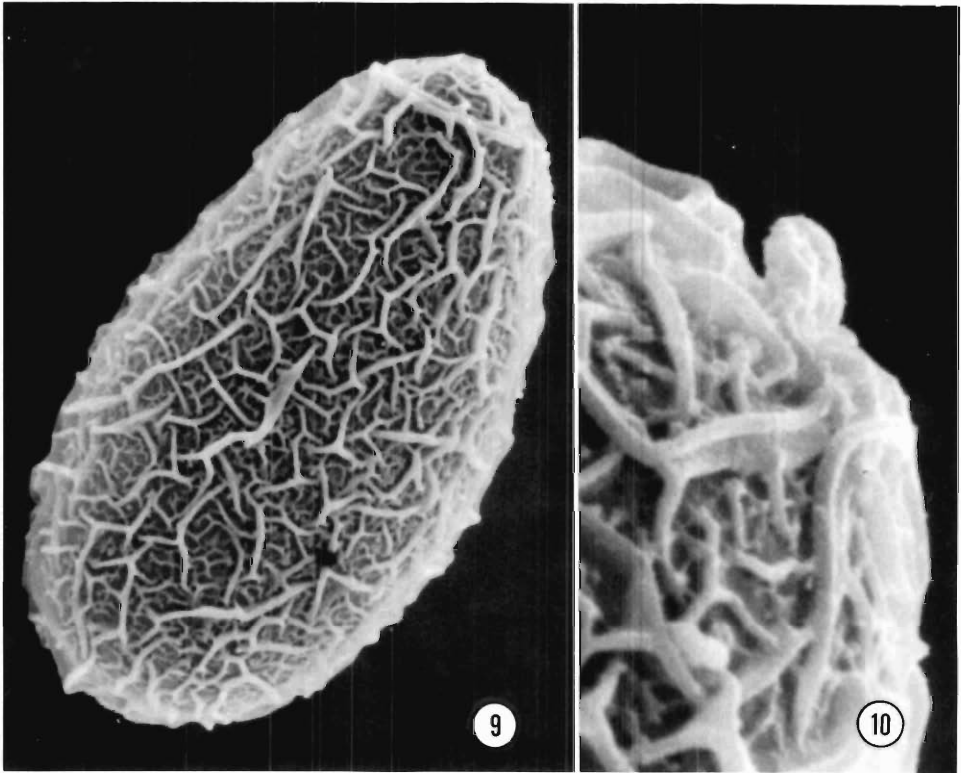
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Figures 1–4. 1. Metacercariae of *Clonorchis sinensis*, showing the outline of attachment to flesh of fish. $\times 1,120$. 2. Excysted metacercaria of *C. sinensis*. Ventral view. $\times 560$. 3. Oral sucker of metacercaria, showing sensory papillae (arrow) and spines. $\times 3,640$. 4. Ventral sucker of metacercaria, showing six rounded protrusions of unknown function, perhaps representing sensory organs. $\times 2,800$.

Figures 5–8. 5. Spines and sensory papillae on ventral surface of metacercaria, about midbody, between suckers. $\times 11,200$. 6. Oral sucker of adult worm, showing spines. $\times 280$. 7. Spines surrounding oral sucker, with shallow depressions where spines probably have been lost. $\times 1,120$. 8. a. Structure on inner surface of oral sucker, showing microvilli different from those on surrounding surface. $\times 5,600$. b. Inside surface of oral sucker of different specimen, showing contraction pattern. $\times 5,600$.







Figures 9, 10. 9. Egg, showing operculum. $\times 3,920$. 10. Abopercular end of egg showing knob. $\times 14,000$.

and is comparable in size to the oral sucker. Six equidistant, distinct protrusions are on the acetabular margin, but are without visible knobs. Two smaller papillae are seen anteriorly and posteriorly on the edge of the ventral sucker. These papillae bear knobs in the center. The surface of the larva is covered with short spines composed of two to four teeth, most frequently three. The spines protrude from the transverse folds of the tegument (Fig. 5), are smaller near the anterior end, and are absent in the pre-acetabular area and at the posterior end of the body. Two rows of round or ovoid sensory papillae are distributed anteroposteriorly on the ventral surface of the body (Fig. 2).

Adult worms have no spines on the surface of the body, except at the rim of the oral sucker. The oral sucker bears groups of stout spines that appear to be easily lost during the course of preparation (Fig. 6). Thus, we could only see them at the margin of the oral sucker, about 10 in each group, but the surface imprints of the lost spines can still be clearly seen in adjacent areas (Fig. 7). The inside surfaces of the oral and ventral suckers are covered with small villi patterned in clusters (Fig. 8a). Occasionally, a larger patch of microvilli is encountered. However, different patterns of contraction can be seen in different specimens (Fig. 8b).

Eggs are mostly pear-shaped with a rough shell surface. Eggs are covered by two layers of ridges differing in size and shape, the layer of smaller ridges lying

underneath the larger one, both crisscrossing each other (Fig. 9). The margin of the operculum and the small knob at the abopercular end are visible (Fig. 10).

Although the morphology of the metacercariae, adult worms, and eggs has been described previously by many authors using light microscopy, several new observations are recorded here. These include the shape and arrangement of the spines of the metacercaria, and the appearance of the spines on the oral sucker of the adult. The papillae on the metacercaria could be seen very distinctly, but their number is somewhat different from that reported by Komiya (1965, loc. cit.). Two morphologically different sensory papillae were seen surrounding the oral sucker; the marks of the lost spines also could be seen there, indicating that these spines are quite weakly attached. The observation that the surface of the buccal cavity is covered by microvilli has not been reported previously. Although the microvilli may serve in food absorption, the surface of the ventral sucker also bears similar structures. The morphology of the eggs was similar to that described by Ishii and Tokunaga (1971, loc. cit.).

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Research Note

Some Digenetic Trematodes of Marine Fish from Virgin Gorda, British Virgin Islands

Between October and December 1978, several species of marine fish were trapped off Virgin Gorda, British Virgin Islands (18°26'29"N to 18°30'21"N and 64°17'50"W to 64°25'56"W) and examined for digenetic flukes. Depth of water in this area ranged from 14.6 to 49.4 m, averaging 27.4 m over a typically flat bottom of coral, volcanic rock, and sand. Trematodes were fixed in 10% buffered formalin, stained in Harris' hematoxylin, cleared in beechwood creosote, and

mounted in Canada balsam. Representative examples of all but one species were deposited in the USNM Parasite Collection, Beltsville, Maryland.

Four of 10 lane snappers, *Lutjanus synagris* (Linnaeus), were infected with *Siphodera vinaledwardsii* (Linton, 1901), a species occurring in shallow-water fishes from Massachusetts to the Caribbean. It was reported in the oyster toadfish, *Opsanus tau* (Linnaeus), at Woods Hole, Massachusetts by Linton (1940, Proc. U.S. Natl. Mus. 88:11–72) and in *L. synagris* and the mutton snapper, *L. analis* (Cuvier), off Punta Arenas, Puerto Rico, by Siddiqi and Cable (1960, N.Y. Acad. Sci. 17:257–368). This species was found in none of 15 *L. analis* examined in this study. Finding it in *L. synagris* off Virgin Gorda constitutes a new locality record. Specimens were deposited as USNM Helm. Coll. No. 77149.

Two of five bluestriped grunts, *Haemulon sciurus* (Shaw), were infected with *Lecithophyllum pyriforme* (Linton, 1910), first described from the spotfin butterflyfish, *Chaetodon ocellatus* Bloch, and the margate, *Haemulon album* Cuvier, off Puerto Real, Puerto Rico. Specimens of *L. pyriforme* from Virgin Gorda were lost, but after they had been prepared as whole mounts and found to agree with the holotype. Finding the species in *H. sciurus* off Virgin Gorda establishes a new locality record.

Five of eight blue tangs, *Acanthurus coeruleus* Bloch and Schneider, were infected with *Pseudodichadena lobata* Yamaguti, 1971, reported from the ocean surgeon, *Acanthurus bahianus* Castelnau, as well as *A. coeruleus* off Mona Island and Puerto Real, Puerto Rico, as *Dichadena acuta* Linton, 1910 by Siddiqi and Cable (1960, loc. cit.). Specimens were deposited as USNM Helm. Coll. No. 77150. This represents a new locality record.

One of five red hinds, *Epinephelus guttatus* (Linnaeus), was infected with *Cainocreadium longisaccum* (Siddiqi and Cable, 1960). Specimens from Virgin Gorda agreed with the holotype from the rock hind, *Epinephelus adscensionis* (Osbeck), taken off Puerto Real, Puerto Rico. Presence of the parasite in *E. guttatus* off Virgin Gorda constitutes a new host and locality record. Specimens were deposited as USNM Helm. Coll. No. 7151.

None of 15 *Lutjanus analis*, 10 nassau groupers, *Epinephelus striatus* (Bloch), or 15 blue runners, *Caranx fuscus* Geoffroy Saint-Hilaire, was positive for digenetic flukes.

The author wishes to express his appreciation to Dr. J. Ralph Lichtenfels, Curator, National Parasite Collection for providing holotype and paratype specimens for examination.

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Research Note

Helminths from the Raccoon, *Procyon lotor litoreus* Nelson and Goldman 1930, on St. Catherines Island, Georgia

Raccoons, *Procyon lotor*, from relatively isolated islands off the Georgia and South Carolina coast have previously been examined for helminths with significantly different results. Jordan and Hayes (1959, J. Parasitol. 45:249-252) reported seven species of gastrointestinal helminths from 100 raccoons on Ossabaw Island, Georgia, and Harkema and Miller (1962, J. Parasitol. 48:333-335) reported 12 genera of helminths from only 16 hosts from Cape Island, South Carolina.

St. Catherines and Ossabaw islands off the coast of Georgia are part of a chain of ecologically similar barrier islands extending from South Carolina to Florida. Although the islands are only 1 mi apart, they are relatively isolated by tidal currents, which probably prohibits migration of most terrestrial mammals between these islands. Habitats on St. Catherines Island are primarily tidal marshes and various forest associations, which are both utilized by raccoons. Migration does occur between these habitats on St. Catherines Island (Harman and Stains, 1979, Am. Mus. Novit. 2679:1-24).

Table 1. Helminths of raccoons, *Procyon lotor*, from St. Catherines Island and Ossabaw Island, Georgia, and Cape Island, South Carolina. Numbers in parentheses indicate number examined.

Parasite	St. Catherines Island (32)			Ossabaw Island* (100)			Cape Island† (16)	
	No. inf.	Range	Mean	No. inf.	Range	Mean	No. inf.	Mean
<i>Atriotaenia procyonis</i> (Chandler, 1942) Gallati, 1956	2	1	1	9	Not given	4	—	—
<i>Physaloptera maxillaris</i> Molin, 1860	11	1-209	40	5	1-4	2	—	—
<i>P. rara</i> Hall and Wigdor, 1918	—	—	—	—	—	—	15	15
<i>Gnathostoma</i> sp.	—	—	—	—	—	—	1	1
<i>G. procyonis</i> Chandler, 1942	10	1-57	17	4	Not given	Not given	—	—
<i>Placoconus lotoris</i> (Schwartz, 1925) Webster, 1956	4	1-2	2	22	1-16	6	1	1
<i>Molineus barbatus</i> Chandler, 1942	—	—	—	21	1-126	10	—	—
<i>Dracunculus insignis</i> Leidy, 1858	1	1	1	—	—	—	—	—
<i>Macracanthorhynchus ingens</i> (v. Linstow, 1879) Meyer, 1932	27	1-931	61	54	1-72	8	4	1.25
<i>Carneophallus turgidus</i> Leigh, 1958	—	—	—	60	3-10,950	1,873	16	10,356

* Data from Jordan and Hayes (1959, op. cit.).
† Data from Harkema and Miller (1962, op. cit.). Ranges not reported.

Thirty raccoons were collected from St. Catherines Island with Tomahawk live traps between 13 January 1975 and 20 May 1975. Two additional animals were trapped in January 1976. All were anesthetized with ketamine hydrochloride and sacrificed with chloroform. Digestive tracts were removed and stomach and colon contents washed separately in a No. 18 U.S. standard sieve (1.0-mm openings) for study of food habits. Filter residue, and stomach and colon contents, were then examined for helminths. Contents of the small intestine were examined intact. Animals were also skinned and examined for *Dracunculus insignis*.

Helminths found, and a comparison of helminths from Cape and Ossabaw islands, are recorded in Table 1. Twenty-seven of 32 raccoons were infected with at least one species of helminth. Representative specimens of *Dracunculus insignis* (77175), *Gnathostoma procyonis* (77176), *Physaloptera maxillaris* (77177), *Placoconus lotoris* (77178), and *Macracanthorhynchus ingens* (77179) have been deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705.

In addition to trematodes listed in the table, *Gynaecotyla adunca* Linton, 1905, *Maritreminoides* sp., *Phagicola diminuta* Stunkard and Haviland, 1924, *P. longa* Ransom, 1920, *Ascocotyle leighi* Burton, 1956, *Lyperosomum sinuosum* Travassos, 1917, *Fibricola texensis* Chandler, 1942, and *Pharyngostomoides procyonis* Harkema, 1942 have also been reported from raccoons of Cape Island (Harkema and Miller, 1962, op. cit.).

Absence of trematodes from St. Catherines Island raccoons is difficult to explain when compared with the large numbers found on Ossabaw and Cape islands. Large numbers of *Macracanthorhynchus ingens* and *Gnathostoma procyonis* on St. Catherines Island also distinguish the parasitofauna of the three islands. One *Dracunculus insignis* was found, which is the first report of this parasite from coastal islands, although it is frequently found on the mainland.

Harkema and Miller (1962, op. cit.) observed many differences when comparing parasites of island and mainland raccoons, and attributed these differences to lack of intermediate hosts on islands, and effects of sunlight, high tide, salty soil, and dry habitat on free-living stages of nematodes. They also presumed little, if any, migration from the mainland, and assumed they had a case of island isolation. It is difficult to explain absence of intermediate hosts from islands close to the mainland and to each other as Harkema and Miller believed was the case, but data from the three studies discussed here may support their assumption of island isolation.

We wish to acknowledge support provided for Dennis M. Harman by the St. Catherines Island Research Program of the American Museum of Natural History, supported by the Edward J. Noble Foundation, and Dr. George Garoian for reviewing the manuscript.

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Research Note

Indications of Seasonal Variation in the Helminth Fauna of the Lesser Prairie Chicken, *Tympanuchus pallidicinctus* (Ridgway) (Tetraonidae), from Northwestern Texas

Pence and Sell (1979, Proc. Helminthol. Soc. Wash. 46:146–149) recovered three helminth species, *Oxyspirura petrowi* Skrjabin, 1929, *Heterakis isolonche* von Linstow, 1906, and *Rhabdometra odiosa* (Leidy, 1887) Jones, 1929, from 10 specimens of the lesser prairie chicken, *Tympanuchus pallidicinctus* (Ridgway) collected from the Texas Panhandle during summer and fall. These authors noted a lack of concentration of dominance of particular helminth species and a basic dissimilarity in the helminth fauna of the lesser prairie chicken and that of the greater prairie chicken, *Tympanuchus cupido* (Linnaeus), collected from other geographic areas. With the subsequent necropsy of an additional 34 lesser prairie chickens during the fall 1977–1978 and spring 1979–1980, the recovery of an additional helminth species, and the apparent seasonal variation noted in the prevalence of certain helminth species, the present study was initiated to examine further the helminth fauna of this host.

Herein, data from seven birds reported on previously (Pence and Sell, 1979, loc. cit.) collected during the fall 1976–1977, 19 birds collected in fall 1979, 10 hosts from spring 1979, and five from spring 1980 were used. All were collected from the same area of Yoakum Co., Texas. Collection procedures, necropsy techniques, and helminth recovery and preparation procedures were as previously described (Pence and Sell, 1979, loc. cit.). For each host, data were available for sex and season.

Chi-square analyses of 2×2 contingency tables were utilized for determining significant relationships between prevalence of each helminth species in the fall and spring collecting periods. The Mann-Whitney *U*-test was used to measure significant correlations of intensities of individual helminth species versus the collecting periods. These analyses were executed as computer programs in the BMDP format (BMDP, Biomedical Computer Programs, P-series, 1979, Univ. California, Berkeley, pp. 248–277, 612–617) on an RSTS V 7.0-08 computer system. Significance was determined at $P \leq 0.05$.

Three nematode and one cestode species were recovered (Table 1). All of 41 lesser prairie chickens were infected with one or more helminth species: 22 of 41 (54%) had one helminth species; 14 of 41 (34%) had two helminth species; and five of 41 (12%) had three helminth species.

There was significantly greater prevalence of *Physaloptera* sp. larvae in the spring sample of lesser prairie chickens than in the fall sample ($\chi^2 = 12.830$, $P = 0.0024$), and of *R. odiosa* in the fall sample than in spring ($\chi^2 = 12.265$, $P = 0.0016$) (Table 1). There were no significant differences in the prevalence of *O. petrowi* ($\chi^2 = 0.581$, $P = 0.6675$) or *H. isolonche* ($\chi^2 = 2.259$, $P = 0.2385$) between spring and fall samples. There was likewise a highly significant increase in the intensity of *Physaloptera* sp. larvae in spring ($P = 0.0006$) and of *R. idiosa*

Table 1. Seasonal variation in helminths of the lesser prairie chicken from northwestern Texas.

Helminth species	Prevalence	Intensity		
	No. infected/ no. examined	%	Range	Mean
Fall				
<i>Oxyspirura petrowi</i>	17/26	65	1–12	5.4
<i>Heterakis isolonche</i>	11/26	42	1–271	66.5
<i>Rhabdometra odiosa</i>	14/26	54	1–29	9.4
<i>Physaloptera</i> sp.*	10/26	38	1	1
Spring				
<i>Oxyspirura petrowi</i>	8/15	53	1–19	3.80
<i>Heterakis isolonche</i>	10/15	67	1–15	17.5
<i>Rhabdometra odiosa</i> †	1/15	7	1	1
<i>Physaloptera</i> sp.*	6/15	40	1–9	3.7

* Larvae.

† Scolex only.

during the fall ($P = 0.0008$) (Table 1). There were no significant differences in the intensities of *H. isolonche* and *O. petrowi* between spring and fall samples. It should be emphasized that these results may be somewhat biased by the low sample size and combining of samples across years (yearly variations due to environmental and dietary changes). Unfortunately, it was not possible to collect larger numbers of hosts during the same year.

Because of the disparity of sample sizes between male ($N = 32$) and female ($N = 9$) hosts and the unavailability of data on host age (juveniles versus adults), these parameters versus helminth prevalence and intensity were not measured statistically.

This is the first record of *Physaloptera* sp. from the lesser prairie chicken. Specimens recovered from this host were all apparently third-stage larvae, and were found in the crop and/or proventriculus. Although *Physaloptera* sp. larvae have been found in the breast musculature of various grouse and quail (Cram, 1932, Trans. 18th Am. Game Conf., pp. 243–247; Broughton, 1937, Univ. Minn. Agric. Exp. Stn. Tech. Bull. 121:1–50; Saugstad, 1943, N. D. Outdoors 5:12–14; Mitchell and Bigland, 1960, J. Wildl. Manage. 24:223; Rollins, 1980, M.S. Thesis, Okla. St. Univ., Stillwater, Okla. 59 pp.), they were not recovered from the breast musculature of the lesser prairie chicken in the present study. *Physaloptera* spp. develop to third-stage larvae in a variety of orthopterans, including cockroaches, field crickets, and camel crickets (Petri and Ameel, 1950, J. Parasitol. 36(Suppl): 40; Sago, 1959, Rev. Bras. Biol. 19:9–12; Zago, 1959, Arq. Zool., São Paulo 11: 59–98). It is suggested that other insects, especially beetles, may serve as first intermediate hosts (Olsen, 1974, Animal Parasites, Their Life Cycles and Ecology. Univ. Park Press, Baltimore. 562 pp.). Lesser prairie chickens presumably become infected as a result of ingesting infected arthropods. Herein they are regarded as paratenic hosts for the *Physaloptera* sp. recovered because only larvae were found.

Seasonal fluctuations in nematode populations are previously reported from other species of gallinaceous game birds (Madsen, 1952, Dan. Rev. Game Biol. 2:1–126). In the ring-necked pheasant, *Phasianus colchicus* (Gmelin), seasonal fluctuations in the prevalence of *O. petrowi* (McClure, 1949, J. Wildl. Manage.

13:304–307) and in the cestode *Choanotaenia infundibulum* (Bolch, 1779) Raillet, 1896 (Gilbertson and Huggins, 1964, J. Wildl. Dis. 8:203–206) are reported. In the latter studies, there was the absence of cestodes in the winter (January and February), and only scolices with immature proglottids were found in the fall (November), respectively. Fluctuating environmental conditions and concurrent changes in diet were suggested as possible factors causing variation in the prevalence of helminth faunas on a seasonal basis (Madsen, 1952, loc. cit.).

In the present study, there appears to be a seasonal variation in the consumption of arthropods (especially grasshoppers, crickets, and beetles) (Doerr, 1980, Effects of Tebuthirum on lesser prairie chicken habitat and food supplies. M.S. Thesis, Texas Tech Univ., Lubbock, Texas. 55 pp.), the presumed intermediate hosts for *Physaloptera* sp. (Zago, 1959, loc. cit.) and *R. idiosa* (Wardle, McLeod, and Radinovsky, 1974, Advances in the Zoology of Tapeworms, 1950–1970. Univ. Minn. Press, Minneapolis. 274 pp.) in the lesser prairie chicken. Arthropods constitute 8, 27, 60, and 65% of the diet during winter, spring, summer, and fall, respectively (Doerr, 1980, loc. cit.). This could account for the dramatic decrease in prevalence of cestodes in the spring versus fall sampling periods, but does not explain the converse relationship with seasonality versus the prevalence of *Physaloptera* sp. larvae. Alternately, other extrinsic factors, such as environmental factors influencing egg survival and longevity, and/or intrinsic factors, such as seasonal physiological changes in the host, could be responsible for the changing composition of the helminth fauna in the lesser prairie chicken. Regardless of the cause of helminth faunal variation at different times of the year, this study emphasizes the necessity of collecting periodic samples throughout the year (or at least a sample during the warm and cool seasons) in order to determine helminth prevalences, intensities, abundance, and concurrent ecological parameters derived from these host data in a host–parasite system.

Representative specimens of identified helminths recovered in this study are deposited in the USNM Helminthological Collection, USDA, Beltsville, Maryland 20705 (Nos. 74690–74692).

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Research Note

**Recovery of *Sarcocystis* Sporocysts from Feces
After Oral Administration¹**

In earlier experiments with avian sarcocystosis, accidental infections occurred among uninfected birds caged separately but housed in the same room with experimentally infected birds (Box and Smith, 1982, J. Parasitol. 68:668–673). *Sarcocystis* species are obligately heteroxenous parasites, alternating between a predator definitive host and its prey, which is the intermediate host. In the normal cycle of this species of *Sarcocystis* (*S. dubonei*; Duszynski and Box, 1978, J. Parasitol. 64:326–329), birds are infected with sporocysts already sporulated when passed in feces by the opossum, *Didelphis virginiana*. Asexual reproduction takes place in the bird, terminating in muscle cysts, which, when eaten, are infective for the opossum. It would, therefore, appear safe to house infected and noninfected intermediate hosts in the same room; viable sporocysts administered to birds should excyst in the gut so that only nonviable sporocysts would be excreted in the feces.

After accidental infections occurred, it was decided to test the validity of this assumption. Sporocysts passed in feces after oral inoculation were tested for viability by reinoculation into budgerigars (*Melopsittacus undulatus*), the most susceptible to sarcocystosis of avian species tested by us (Box and Smith, 1982, loc. cit.).

Initially, two budgerigars were given 20×10^3 sporocysts each, caged together, and all feces collected at intervals of 24, 48, 72, and 96 hr PI (postinfection). A single sporocyst was found by sugar flotation of the feces collected at 24 hr, but none was found in the remaining samples. As the inoculated sporocyst dose was low compared to some doses used when accidental infections occurred, a further experiment was set up with larger inocula and with four host species of varying susceptibilities to the infection (Table 1). These included the budgerigar, canary (*Serinus canarius*), white mouse (*Mus musculus*), and chicken (*Gallus gallus*). Both budgerigars and canaries are susceptible to the asexual stages of this species of avian *Sarcocystis*, whereas the chicken is not (Box and Smith, 1982, loc. cit.). In addition, unpublished observations indicate that the mouse is not susceptible to infection with this *Sarcocystis* species, but excystation has been observed in the gut of both chick and mouse. Sporocysts in the inoculum were from gut scrapings of an opossum infected by feeding it experimentally infected canaries 12–22 days earlier. Cages or cage bottoms (in the case of the canary and budgerigar) were autoclaved and washed between each collection. Three different methods were used to estimate number of sporocysts in the feces collected. A simple smear was made of the fecal collection and if sporocysts were scarce, the total feces collected for a sampling period was examined after flotation; if they were mod-

¹ This work was supported in part by Public Health Service Grant DHHS 5R01 AI 15945 from the National Institute of Allergy and Infectious Diseases.

Table 1. Numbers* of sporocysts recovered after oral inoculation.

Recipient	Budgerigar	Canary	Chick	Mouse 1	Mouse 2
Sporocyst dose × 10 ⁶					
Total	18	8	10.5	6	17
Per g body wt	0.5	0.5	0.25	0.25	0.5
Recovery in feces PI					
0–24 hr	2.3 × 10 ⁶	70,000	3.8 × 10 ⁶	90,000	858,000
24–48 hr	264,000	62	576	4	
48–72 hr	11	0	9	1	
72–96 hr	2	0			
Gut scrapings (necropsy, PI)	0 (96 hr)	0 (96 hr)	0 (72 hr)	0 (72 hr)	437,000 (24 hr)

* Count after flotation except that numbers × 10⁶ were counted by hemacytometer and those × 10³ were counted by micropipette drop.

erately plentiful, a 20 µl drop on a slide was counted under a cover glass; and if sporocysts were numerous, a hemacytometer count was made of the sporocysts.

After the last fecal collection, the animals were necropsied and scrapings of the entire intestine were digested with pepsin and trypsin (Box and Smith, 1982, loc. cit.). Sporocysts were counted in the digest residue, or if none were found, a sugar flotation was done.

A relatively large number of sporocysts was passed in the feces in the first 24 hr PI of all four animal species, and also in budgerigar feces collected from 24 to 48 hr PI (Table 1). By 72 and 96 hr PI, sporocysts were absent or only detectable at a low level. Gut scrapings were negative, except for those from the mouse necropsied at 24 hr PI.

To ascertain whether the sporocysts were viable, they were cleaned by centrifugation in a sucrose gradient and were given orally to six budgerigars in amounts equal to about 26–56 sporocysts/g body wt, a dose usually lethal to these birds. Samples given included 24-hr collections from the budgerigar, chick, and mice; 48-hr collections from the budgerigar; and sporocysts in gut scrapings from Mouse #2 at 24 hr PI. All six of the budgerigars became infected, and four died between 4 and 6 wk PI. The two surviving birds received sporocysts recovered from chick feces and mouse gut scraping after 24 hr; they were necropsied at 11 and 20 wk PI. All six recipient birds had numerous muscle cysts as well as preceding stages (tachyzoites) in lungs and other organs. Three uninoculated budgerigars kept in the same room remained healthy, and no muscle infection was seen at necropsy. It was concluded that most of the recovered sporocysts were viable, because after their inoculation the mortality response of budgerigars was similar to that observed in previous experiments in which sporocysts directly from opossum gut scrapings were used. The latter experiments included eight budgies given 50–65 sporocysts/g body wt. All died of the infection, five at 4 wk PI, one each at 5 and 6 wk PI, and one at 24 wk PI.

The ability of viable sporocysts to pass through the gut corresponds to observations of Dubey and Frenkel (1973, J. Parasitol. 59:505–512) on oocysts of *Toxoplasma*. They found that about 10% of orally administered oocysts were

passed in feces of experimentally infected mice within 24 hr and less than 0.01% after 24 hr. About 90% of the oocysts were infectious for other mice.

Viable sporocysts may not all excyst in a susceptible host because not all are exposed equally to digestive enzymes as they pass through the gut. It is possible that in the present experiment, the large proportion of sporocysts (78%) that were still inside the oocyst in the inoculum may have enhanced passage of viable sporocysts in feces. Usually sporocysts recovered from feces of the definitive host are free of the oocyst wall, but in gut scrapings of the same host, they are more commonly still inside the oocyst wall (Box and Smith, 1982, loc. cit.). It is believed that the sporocysts in the inoculum were fully mature because they were obtained from gut scrapings 12–22 days after the opossum was fed infected canaries, an adequate time for sporulation (Box and Duszynski, 1980, J. Wildl. Dis. 16:209–215).

Sporocysts in the environment, whether from experimentally infected intermediate hosts or from normal definitive hosts, can interfere with experimental results, especially in view of their ability to persist in the environment. Smith and Frenkel (1978, J. Parasitol. 64:315–319) found that cockroaches could transmit sporocysts of *Sarcocystis muris* to the mouse intermediate host for at least 20 days after fecal excretion by the cat definitive host. They found that sporocysts in dried cat feces also remained infective for 20 days. Bergler et al. (1980, Berl. Munch. Tierarztl. Wochenschr. 93:288–293) found sporocysts of *Sarcocystis* of pigs and goats (*S. suicanis*, *S. suihominis*, and *S. capreolicanis*) capable of surviving 7 wk in feces.

Experimental evidence of the ability of viable oocysts or sporocysts to pass through the intestine without excysting is, therefore, of obvious importance in experimental infections and a phenomenon that may also aid in dispersal of the organism in nature.

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Research Note

Effects of Viscous Agar on In Vitro Pairing of *Echinostoma revolutum* (Trematoda) Adults

Intraspecific pairing of trematodes in vitro has been reported for five species: *Amblosoma suwaense* (Fried and Robinson, 1981, Parasitology 82:225–229), *Echinostoma revolutum* (Fried, Tancer, and Fleming, 1980, J. Parasitol. 66:1014–1018; Fried and Wilson, 1981, Proc. Helminthol. Soc. Wash. 48:243–245), *Haematoloechus medioplexus* (Fried and Leiby, 1982, Parasitology 84:375–380), *Leu-*

cochloridiomorpha constantiae (Fried and Roberts, 1972, J. Parasitol. 58:88–91), and *Zygocotyle lunata* (Fried and Wilson, 1981, loc. cit.). Observations in the aforementioned studies were made on two worms placed 2 cm apart in 5.5-cm-diameter Petri dishes containing a solid agar substratum and a Locke's solution overlay.

The purpose of this study was to use a viscous agar overlay to test the effects of the overlay and gravity on the intraspecific pairing of *Echinostoma revolutum* adults.

Echinostoma revolutum adults from experimentally infected domestic chickens (Fried and Weaver, 1969, Proc. Helminthol. Soc. Wash. 36:136–138) were washed rapidly in three changes of cold sterile Locke's solution, and used for pairing studies within 10 min of host necropsy. To study pairing, 5.5-cm Petri dishes were coated with a 1-mm substratum of solid agar (1.1% Purified Agar, Oxoid Limited, Hampshire, England) and a viscous overlay of either 0.5 or 0.7% agar. The viscous agar overlays were prepared by adding either 0.5 or 0.7 g of Purified Agar to 100 ml of Locke's solution just brought to a boil. Each agar solution was poured into Petri dishes coated with a solid agar substratum. The viscous agar was allowed to gel for 5 min at 5°C. Dishes were then placed in an incubator at 39°C for pairing studies. Two worms were placed 2 cm apart in each dish, and those in contact or within 5 mm of each other were considered paired. Observations were made at various intervals up to 2 hr postinoculation of worms. To test the effect of gravity on pairing, worms were placed 2 cm apart in dishes containing a 0.7% agar overlay. Each dish was covered and placed vertically in 11 × 4-cm egg cartons and maintained at 39°C for 2 hr. All worms were alive and active after 2 hr, at which time the experiments were arbitrarily terminated.

In studies that matched a single worm with a dead worm or an inert object, pairing did not occur. Worms paired in all viscous agar solutions as seen previously in Locke's solution overlays (Fried, Tancer, and Fleming, 1980, loc. cit.). In 20 trials using dishes with the 0.5% agar overlay, 10 worm pairs (50% pairing) were seen at 2 hr. In a previous study using ovigerous worms and a Locke's overlay, worm pairing at 2 hr was 54% (Fried, Tancer, and Fleming, 1980, loc. cit.).

Gravity had no apparent effect on pairing in the 0.7% agar overlay. In 20 trials using vertical dishes and the 0.7% agar overlay, nine worm pairs (45% pairing) were seen at 2 hr, and worms paired in all locations in the dish.

In our study worms migrated either at the interface of the agar substratum and viscous overlay, or through the viscous overlay. In studies on chemotaxis of nematodes (Ward, 1978, in *Taxis and Behavior*. Chapman Hall, London), worms migrated through viscous agar and produced tracks. Regardless of the viscosity of the agar overlay in our study, *E. revolutum* adults did not leave clear tracks.

In this study worms failed to show copulatory behavior, an observation consistent with a previous study on *E. revolutum* by Fried, Tancer, and Fleming (1980, loc. cit.) and a study on *L. constantiae* by Fried and Roberts (1972, loc. cit.).

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MINUTES

Five Hundred Forty-Ninth Through Five Hundred Fifty-Sixth Meetings

549th Meeting: Uniformed Services University of Health Sciences (USUHS), Bethesda, Maryland, 15 October 1982. The following slate of officers was presented: Milford N. Lunde (President), Sherman S. Hendrix (Vice President), Michael D. Ruff (Corresponding Secretary-Treasurer), Raymond V. Rebois (Recording Secretary). The 1982 Anniversary Award was presented to Lloyd E. Rozeboom. A moment of silence was observed in honor of the following deceased members: Rex Allen, Clay Huff, Willard Wright, and Gordon Ball. LTC Bryce C. Redington presided over the following papers: "Mechanical Transmission of *Trypanosoma rhodesiense* by Tsetse Flies," John B. Gingrich; "Temperature-induced *In Vitro* Transformation of *Leishmania mexicana*," Kenneth W. Hunter; "*Plasmodium falciparum*: Scanning for Cell Surface Alterations," Elizabeth Peterson; and "Extraordinary Parasite Densities Attained by *Plasmodium vivax* in Ethiopia," Joseph C. Armstrong.

550th Meeting: Animal Parasitology Institute, USDA, Beltsville, Maryland, 12 November 1982. G. F. Otto reported that the Finance Committee estimated there would be a \$2,500 deficit in the 1982 budget. Assuming membership stayed about the same and with the increased 1983 membership dues (to \$15.00) the Society may break even in 1983 and 1984. In order to prevent future deficits the Executive Committee approved: (1) optional raising of the publications page charges to \$30.00 in 1983; (2) a membership tear-out page to be inserted when there is an unused page in the "Proceedings"; (3) the price of reprints be increased 25%; and (4) the limit on the number of published pages in each volume of the "Proceedings" be removed. The slate of officers presented at the 549th meeting was unanimously elected. J. Ralph Lichtenfels was elected Editor of the "Proceedings" to succeed A. J. Haley. M. D. Ruff presided over the following papers: "Superoxide Dismutase in *Trichinella spiralis*," Marcia Rhoads; "Studies on Immediate Hypersensitivity in Swine Trichinosis," Carol Durham; "Studies on the Invasion of Cultured Cells by *Eimeria* Sporozoites," Patricia C. Augustine and Harry Danforth; and "The Action of Albendazole in *Fasciola hepatica*-infected Ruminants," R. H. Fetterer.

551st Meeting: Plant Protection Institute, USDA, Beltsville, Maryland, 3 December 1982. No business was discussed. L. S. Diamond announced he would present the gavel to the newly elected President Milford N. Lunde prior to the next meeting. R. V. Rebois presided over the following papers: "The Current Status of the Pine Wood Nematode, *Bursaphelenchus xylophilus*," William R. Nickle; "Sterol Metabolism in *Caenorhabditis elegans*," David J. Chitwood; and "Genetic Basis for the Separation of Two Races of *Radopholus similis*," Robin N. Huettel.

552nd Meeting: National Institutes of Health, Laboratory of Parasitic Diseases, Bethesda, Maryland, 14 January 1983. It was announced that R. Barclay McGhee

had passed away. President M. N. Lunde announced: (1) Willis A. Reid was appointed Member at Large to complete the term vacated by L. D. Hendricks; and (2) appointees to the new Membership Committee are L. S. Diamond (Chairman), N. D. Pacheco, and M. N. Lunde. Franklin Neva presided over the following papers: "Results of a Field Test in West Africa of a New RIA for Malaria Sporozoites," Frank Collins; "Falciparum Malaria: Partial Characterization of Knobs on Infected Erythrocytes," James Leech; "Immunologic Determinants of Onchocerciasis," Henry Francis, Eric Ottesen, and Kwablah Awadzi; and "Interactions of Certain Bacteria, Especially *Salmonella*, with Schistosomes *In Vitro* and *In Vivo*," Carmen Tuazon.

553rd Meeting: Naval Medical Research Institute, Bethesda, Maryland, 18 February 1983 (co-sponsored by Oxford Biological Laboratory, National Marine Fisheries Service). M. D. Ruff presented the Treasurer's Report. Aaron Rosenfield and Wilton Vannier presided over the following papers: "The Comparative Morphology of Pathogenic and Nonpathogenic *Acanthamoeba hatchetti*," Shawn Bodammer and Thomas K. Sawyer; "Changes in Host Tissue Energy Metabolism Due to Invasion of Parasitic Worms," Krystyna Boczon; "Autoradiographic Analysis of Acquired Resistance to *Schistosoma mansoni* Infection," David Dean; and "One Step *In Vitro* Growth of Synchronized Cultures of *Plasmodium falciparum*," Werner Zolg.

554th Meeting: Walter Reed Army Institute of Research, Washington, D.C., 11 March 1983. J. W. Bier announced that the Food and Drug Administration would have six to 15 Summer Internships for graduate students available this summer in Washington, D.C. M. N. Lunde announced the Executive Committee had approved the recommendations of the Business Advisory Committee concerning requests put forth by Allen Press that: (1) the Society accept the Allen Press recommendation that the Society maintain the fiscal books and Allen Press would continue to mail the Journal, maintain the mailing list, make deposits, receive checks, mail dues and subscription notices, and collect dues; (2) Allen Press computerize the Society mailing list so it can be cross computerized with other Society lists such as ASP. The cost would be a one-time charge of \$0.65 per member for a total cost of approximately \$650.00. As a result the charge for processing dues and subscriptions will be reduced from \$3.00 and \$4.00, respectively, to \$2.00 and \$3.00 for an annual savings to the Society of approximately \$1,000.00; Allen Press will handle the cost of copyrighting the Proceedings, provided it is not already copyrighted, at a cost of \$20.00 per issue. M. D. Ruff presented the 1982 Audit Committee Report. A motion was made, seconded, and approved to accept the report. LTC Willis A. Reid, Jr. presided over the following papers: "Analysis of Clones and Human Isolates of *Trypanosoma brucei rhodesiense* with Monoclonal Antibodies," Donald E. Burgess, Gary Campbell, Bruce Welde, and Klaus Esser; "Studies on the *In Vitro* Cultivation of *Dipetalonema viteae* Third Stage Larvae," Eileen Franke; "Dot Enzyme-linked Immunosorbent Assay (Dot-ELISA): A Microtechnique for the Rapid Diagnosis of Visceral Leishmaniasis," Michael G. Pappas, Ruta Hajkowski, and Wayne T. Hockmeyer; and "In Vitro Modulation of Surface Antigens on *Schistosoma mansoni* as a Result of Benzyl Alcohol Treatment," Robert E. Miller.

555th Meeting: The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland, 15 April 1983. No business was discussed. Everett L. Schiller presided over the following papers: "Monoclonal Antibodies Against Somatic Antigens of *Dirofilaria immitis*," Alan Scott, Steven Showalter, and David Levy; "Experimental Pathology of *Eustrongylides* in Rabbit," Djamshid Shirzian; "Prevalence of *Toxocara cati* Infections in Baltimore Cats and the Occurrence of *Toxocara Ova* in Urban Gardens," James Childs; "*Leishmania donovani*: Solubilization and Partial Purification of an Unusual Nucleotidase," Gary Zlotnick, Dennis Dwyer, and Michael Gottlieb.

556th Meeting: The University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania, 14 May 1983. President M. N. Lunde announced the following: effective in 1984 the annual subscription rate for the "Proceedings" would be raised from \$22.00 to \$30.00 domestic and from \$24.00 to \$33.00 for all other subscribers. Saturday, 6 August 1983, the Helminthological Society of Washington would have a picnic at Bethesda, Maryland. Frank G. Tromba had been nominated Life Member of the Society. The nomination was approved by the Society members present. The symposium title was "Parasites: Do Methods for Satisfactory Control Exist?" Gerhard S. Schad presided. The following papers were presented: "Onchocerciasis," Ed W. Cupp; "Schistosomiasis," Donald B. Hoffmann; and "Malaria," Franklin A. Neva.

The following 41 new members were elected at the meetings indicated: *549th:* David J. Bergeron, Janine N. Caira, Marc D. Castle, Lee Cohen, L. K. Eveland, D. N. Fotedar, David R. Glen, Murray J. Kennedy, Wieslaw J. Kozek, David A. Leiby, Mike Moser, John Cowardine Pearson, Vishnu-Priya Sneller, and E. Tressalti. *550th:* Robin N. Huettel, M. Ali Ozcel, T. Michael Stock, and Douglas L. Varner. *551st:* Martin Adamson. *553rd:* Louis C. Gasbarre, Robert G. Hamilton, Eileen D. Franke, Michel Charles Luc, Robert E. Miller, Milan Trpis, and Douglas B. Woodmansee. *554th:* Ann M. Adams, Krystyna Boczon, and Chris Lambros. *555th:* David J. Chitwood, Seung-Yull Cho, Terry A. Dick, R. Alan Henn, James E. Joy, and Mustafa M. Ramadan. *556th:* James G. Baldwin, William M. D. Boto, Rodney H. Duvall, Lynne C. Miller, Richard T. O'Grady, and Alan L. Scott.

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Norman R. Stoll	1976	Bernard Bezubik	1980
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CHARTER MEMBERS 1910

W. E. Chambers	Philip E. Garrison	Maurice C. Hall	Charles A. Pfender
Nathan A. Cobb	Joseph Goldberger	Albert Hassall	Brayton H. Ransom
Howard Crawley	Henry W. Graybill	George F. Leonard	Charles W. Stiles
Winthrop D. Foster			

LIFE MEMBERS

Maurice C. Hall	1931	May Belle Chitwood	1975
Albert Hassall	1931	Carlton M. Herman	1975
Charles W. Stiles	1931	Lloyd E. Rozeboom	1975
Paul Bartsch	1937	Albert A. Taylor	1975
Henry E. Ewing	1945	David R. Lincicome	1976
William W. Cort	1952	Margaret A. Stirewalt	1976
Gerard Dikmans	1953	Willard H. Wright	1976
Jesse R. Christie	1956	Benjamin Schwartz	1976
Gotthold Steiner	1956	Mildred A. Doss	1977
Emmett W. Price	1956	Everett E. Wehr	1977
Eloise B. Cram	1956	Marion M. Farr	1979
Gerald Thorne	1961	John T. Lucker, Jr.	1979
Allen McIntosh	1963	George W. Luttermoser	1979
Edna M. Buhner	1963	John S. Andrews	1980
Benjamin G. Chitwood	1968	Leo A. Jachowski, Jr.	1981
Aurel O. Foster	1972	Kenneth C. Kates	1981
Gilbert F. Otto	1972	Francis G. Tromba	1983
Theodor von Brand	1975		

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